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# בקשה לפטנט
# Application For Patent

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מולקולות DNA ותאים שעברו טרנספקציה על ידן

# DNA MOLECULES AND CELLS TRANSFECTED THEREWITH

Yeda Research and Development Co. Ltd.
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Bar-Ilan Research and Development Co. Ltd.

# DNA MOLECULES AND CELLS TRANSFECTED THEREWITH

## FIELD OF THE INVENTION

The present invention relates to cDNA molecules encoded by T cell receptor (TCR) genes and to antisense DNA molecules of said cDNAs and their use in the modulation of mesenchymal cell growth and cell functions. It further relates to the novel proteins, or peptides encoded by these transcripts, and uses thereof.

## DESCRIPTION OF THE RELATED ART

MHC-restricted T cells express heterodimeric surface protein receptors (αβTCR) co-localized with up to five additional non-variant membrane receptors (Strominger, 1989; Abbas et al., 1994; Jameson et al., 1995). This TCR complex specifically binds processed peptide antigens associated with MHC molecules. The interactions of TCR with MHC bound peptides on various target cells may have consequences both in terms of T cell proliferation and in activation of effector mechanisms leading to target cell killing, graft rejection, and other biological effects.

MHC class I gene products are widely expressed by various cell types while MHC class II molecules are expressed constitutively or are inducible in fewer, yet rather diverse cell types, such as dendritic cells, B lymphocytes, macrophages and vascular endothelial cells. By contrast, the T cell receptor complex is thought to be expressed solely by T cells, which further possess complicated signaling cascades as well as specific enzymes engaged in TCR gene rearrangement. Thus, recognition of MHC presented peptides seems to be a highly specific T cell function.

Functional TCR α and β chain genes, which are capable of being expressed as polypeptides, are normally present only in cells of the T lymphocyte lineage. These functional TCR genes are formed by somatic rearrangement of germline gene segments. Each TCR locus consists of variable (V), joining (J), and constant (C) region genes, and the β chain locus contains diversity (D) gene segments. In mice there are 20 to 30 Vβ gene segments that are located 5' of the two clusters of C and J segments. There is a single Cα gene associated with a large 5' cluster of up to 50 different J segments and about 75 Vα segments. There is a large region of intervening DNA between Vα and Jα exons, which includes the entire TCR ∂ chain locus. During maturation of T cells in the thymus, the TCR segments are rearranged in a defined order, resulting in the formation of functional

TCRα and β genes in which V, D, J and C segments are in close proximity to each other.

The β chain locus rearranges prior to the α locus. The primary transcripts contain noncoding intronic sequences between the VDJ and C genes, which are later spliced out. The functional T cell receptor is comprised of 2 polypeptides: the α chain is a 40 to 60 kD acidic glycoprotein, and the β chain is a 40 to 50 kD uncharged or basic glycoprotein. The V and C regions of α and β chains form intrachain disulfide bond loops, which might contribute to the formation of a tertiary structure and are presented on the cell membrane. The C region contains the transmembrane domain and a short cytoplasmic tail thought to be too small to have intrinsic signal transducing properties.

T cells (Qian et al., 1993; Yoshikai et al., 1984) as well as B cells (Calman and Peterlin, 1986) express a series of incomplete transcripts of TCRα and β, that vary in size and structure. These transcripts may be out of frame or their sequence may contains many stop codons. In some cases mRNAs encoding the constant region flanked by an upstream spliced J segment were identified. In one case such a transcript of human TCRβ, which contains an in-frame codon for methionine has been reported (Fagioli et al., 1991). However, no evidence for the existence of a protein encoded by these transcripts in T cells has been documented. TCR transcripts have also been reported in cell lineages other than T or B lymphocytes. Thus, TCRα mRNA was identified in murine kidney (Madrenas et al., 1992; Madrenas et al., Transplanation Proceedings 23: 837, 1991; Madrenas et al., 1994). A recent study identified in ephithelial tumor cells a partial TCRγ chain mRNA, lacking the V region. This mRNA encodes a 7 kDa protein, TARP, which is translated form a alternate reading frame and is therefore not homologous to the TCRγ protein (Essand et al., 1999; Wolfgang et al., 2000). No evidence for TCRab or TCRδ transcripts or proteins was found in this study. It is therefore generally accepted that TCRβ transcripts are not found outside of the lymphocyte lineage and that TCR protein expressed at the cell surface is a specific T cell trait.

Mesenchymal cells play a central role in embryogenesis by directing organogenesis. In the adult organism, tissue remodeling, such as that occurring in wound healing, are initiated by mesenchymal fibroblasts. The study of regulation hemopoiesis demonstrated that blood cell formation is locally regulated by stromal mesenchyme (Zipori, 1989; Zipori et al., 1989; Zipori, 1990; Weintraub et al., 1996). Indeed, bone marrow-derived primary stroma as well as a variety of mesenchymal cells lines derived from primary bone marrow cultures exhibit an in vitro capacity to support hemopoiesis

and, upon transplantation, promote the formation of bone and hemopoietically active tissue in vivo at the site of transplantation. The molecules that mediate the instructive stromal activities have been shown to be a variety of cytokines and adhesion molecules. However, the molecules identified thus far cannot account for the wide spectrum of stromal cell functions and certainly do not explain stroma organization, stem cell renewal and other vital stromal functions.

Mesenchymal cells from the bone marrow are well known to be obligatory for the maintenance and renewal of hemopoietic stem cells in vitro, and these cells are critical for the maintenance of hemopoiesis in vivo. This function of the mesenchyme is not restricted to blood cells. In fact, every tissue and organ is composed of a stromal mesenchyme support that interacts with the other, tissue specific cell types. Thus, the growth and differentiation of cells within different tissues, and the development of tumors, are all dependent on mesenchymal functions.

There is an unmet need for and it would be advantageous to have polypeptide markers for mesenchymal cells that are involved in control of proliferation and differentiation of hemopoietic stem cells. Citation of any document herein is not intended as an admission that such document is pertinent prior art, or considered material to the patentability of any claim of the present application. Any statement as to content or a date of any document is based on the information available to the applicant at the time of filing and does not constitute an admission as to the correctness of such a statement.

## SUMMARY OF THE INVENTION

The present invention relates to new mRNAs transcripts and encoded proteins, which are short versions of $\alpha$ and $\beta$ chains of the T cell receptor (TCR) as detailed herein below, and to uses of these molecules.

The present invention relates, in one aspect, to a cDNA molecule encoded by a T cell receptor (TCR) gene, said cDNA molecule lacking V region sequences and comprising a constant (C) domain and a joining (J) region sequence, and a 5' intronic J sequence upstream said J region sequence including an in-frame methionine codon.

The novel polynucleotide sequences disclosed herein and the corresponding proteins, polypeptides or peptides encoded by these polynucleotide sequences may be derived from any mammalian species including human genetic material.

According to another aspect of the present invention it is now disclosed that mesenchymal cells express variants of the T cell receptor, and that these TCR variants are unique to mesenchymal cells.

According to yet another aspect of the present invention these mesenchymal truncated TCR variants are involved in cell growth control.

According to a further aspect of the present invention the mesenchymal TCR variants are either directly or indirectly is involved in the regulation of stem cell growth and differentiation.

In one embodiment of the invention, the cDNA molecule is encoded by a mouse TCRβ gene. The joining (J) gene sequence may be selected from, but is not limited to, Jβ2.1 and Jβ2.6.

According to this embodiment of the invention, the joining (J) gene sequence may be Jβ2.1 and said 5' intronic J sequence including an in-frame methionine codon codes for a peptide of the sequence M E N V S N P G S C I E E G E E R G R I L G S P F L as depicted in Fig. 4. In an alternative, the joining (J) gene sequence is Jβ2.6 and said 5' intronic J sequence including an in-frame methionine codon codes for a peptide of the sequence M G E Y L A E P R G F V C G V E P L C as depicted in Fig. 4. The cDNA molecule in a preferred embodiment has the nucleotide sequence depicted in Fig.1.

In another embodiment of the invention, the cDNA molecule is encoded by a TCRα gene. In this case, the joining (J) gene sequences are selected from, but not limited to, JαTA31, JαTA46, JαNew05, JαS58, JαNew06, JαNew08, JαLB2A, JαDK1, and JαTA39.

According to this embodiment of the invention, the cDNA molecule having a 5' intronic J sequence including an in-frame methionine codon is selected from the group consisting of:

(i) the intronic JαTA31 gene sequence coding for the peptide:
M A W H

(ii) the intronic JαTA46 gene sequence coding for the peptide:
M E A G W E V Q H W V S D M E C L T V

(iii) the intronic JαTA46 gene sequence coding for the peptide:
M E C L T V

(iv) the intronic JαNew05 gene sequence coding for the peptide:
M T V

(v) the intronic JαS58 gene sequence coding for the peptide:

M C G S E E V F V V E S A

(vi) the intronic JαNew06 gene sequence coding for the peptide:

M A C Y Q M Y F T G R K V D E P S E L G S G L E L S Y F H T G G S S Q A V G L F I E N M I S T S H G H F Q E M Q F S I W S F T V L Q I S A P G S H L V P E T E R A E G P G V F V E H D I

(vii) the intronic JαNew06 gene sequence coding for the peptide:

M Y F T G R K V D E P S E L G S G L E L S Y F H T G G S S Q A V G L F I E N M I S T S H G H F Q E M Q F S I W S F T V L Q I S A P G S H L V P E T E R A E G P G V F V E H D I

(viii) the intronic JαNew06 gene sequence coding for the peptide:

M I S T S H G H F Q E M Q F S I W S F T V L Q I S A P G S H L V P E T E R A E G P G V F V E H D I

(ix) the intronic JαNew06 gene sequence coding for the peptide:

M Q F S I W S F T V L Q I S A P G S H L V P E T E R A E G P G V F V E H D I

(x) the intronic JαNew08 gene sequence coding for the peptide:

M W W G L I L S A S V K F L Q R K E I L C

(xi) the intronic JαLB2A gene sequence coding for the peptide:

M V G A D L C K G G W H C V

(xii) the intronic JαDK1 gene sequence coding for the peptide:

M R E P V K N L Q G L V S

(xiii) the intronic JαTA39 gene sequence coding for the peptide:

M E V Y E L R V T L M E T G R E R S H F V K T S L; and

(xiv) the intronic JαTA39 gene sequence coding for the peptide:

M E T G R E R S H F V K T S L.

According to an alternative and more preferred embodiment, the novel intronic sequences and their corresponding peptides may be derived from human genetic material. Any known sequences, such as the joining segment of human Jβ 2.3 gene, are explicitly excluded from the claimed novel sequences.

According to this embodiment of the invention, the cDNA molecule having a 5' intronic J sequence including an in-frame methionine codon is selected from the group consisting of:

1) the intronic Jβ2.3 gene sequence coding for the peptide:

M G L S A V G R T R A E S G T A E R A A P V F V L G L Q A V

2) the intronic Jα2 gene sequence coding for the peptide

M

3) the intronic Jα3 gene sequence coding for the pep tide:

M L L W D P S G F Q Q I S I K K V I S K T L P T

4) the intronic Jα6 gene sequence coding for the peptide:

M L P N T M G Q L V E G G H M K Q V L S K A V L T V

5) the intronic Jα6 gene sequence coding for the peptide:

M G Q L V E G G H M K Q V L S K A V L T V

6) the intronic Jα6 gene sequence coding for the peptide:

M K Q V L S K A V L T V

7) the intronic Jα8 gene sequence coding for the peptide:

M S E C

8) the intronic Jα9 gene sequence coding for the peptide:

M A H F V A V Q I T V

9) the intronic Jα11 gene sequence coding for the peptide:

M G I C Y S

10) the intronic Jα13 gene sequence coding for the peptide:

M K R A G E G K S F C K G R H Y S V

11) the intronic Jα14 gene sequence coding for the peptide:

M L T T L I Y Y Q G N S V I F V R Q H S A

12) the intronic Jα24 gene sequence coding for the peptide:

M Q L P H F V A R L F P H E Q F V F I Q Q L S S L G K P F C R G V C H S V

13) the intronic Jα25 gene sequence coding for the peptide:

M

14) the intronic Jα31 gene sequence coding for the peptide:

M G F S K G R K C C G

15) the intronic Jα36 gene sequence coding for the peptide:

M K K I W L S R K V F L Y W A E T L

16) the intronic Jα40 gene sequence coding for the peptide:

M G K V H V M P L L F M E S K A A S I N G N I M L V Y V E T H N T V

17) the intronic Jα40 gene sequence coding for the peptide:

M P L L F M E S K A A S I N G N I M L V Y V E T H N T V

18) the intronic Jα40 gene sequence coding for the peptide:

M E S K A A S I N G N I M L V Y V E T H N T V

19) the intronic Jα40 gene sequence coding for the peptide:

M L V Y V E T H N T V

20) the intronic Jα41 gene sequence coding for the peptide:

M E E G S F I Y T I K G P W M T H S L C D C C V I G F Q T L A L I G I I G E G T W W L L Q G V F C L G R T H C

21) the intronic Jα41 gene sequence coding for the peptide:

M T H S L C D C C V I G F Q T L A L I G I I G E G T W W L L Q G V F C L G R T H C

22) the intronic Jα44gene sequence coding for the peptide:

M E S Q A T G F C Y E A S H S V

In another aspect, the invention relates to antisense DNA molecules of any of the cDNA molecules of the invention described above.

The invention further relates to expression vectors comprising the cDNA and antisense molecules of the invention, and to host cells, particularly mammalian cells, comprising said vectors. In one preferred embodiment the host cells are transfected mesenchymal human cells.

The cDNA of the invention can be used to transfect mesenchymal human cells for inducing mesenchymal cell growth. Thus the invention relates to compositions comprising said transfected mesenchymal human cells for use in disorders requiring induction of mesenchymal cell growth, such as wound healing.

The invention further relates to a method for inducing mesenchymal cell growth comprising the step of administering to a subject in need thereof autologous transfected mesenchymal human cells comprising a cDNA molecule according to the invention, in an amount effective to induce mesenchymal cell growth. This method is preferably applicable for enhanced wound healing.

The antisense DNA molecules of the invention can be used to transfect mesenchymal human cells for inhibiting or suppressing mesenchymal cell growth. Thus the invention relates to compositions comprising said transfected mesenchymal human cells for use in disorders requiring inhibition or suppression of mesenchymal cell growth, such as in carcinomas.

The invention further relates to a method for suppressing mesenchymal cell growth comprising the step of administering to a subject in need thereof autologous transfected mesenchymal human cells comprising an antisense DNA molecule of the invention, in an amount effective to suppress mesenchymal cell growth, such as for suppression of carcinomas.

The invention further relates to a polypeptide encoded by a DNA molecule of the invention. In one embodiment, said polypeptide is a protein capable of being expressed on the cell surface or intracellularly, and is encoded by the nucleotide sequence depicted in Fig. 1, more preferably, the protein of the amino acid sequence depicted in Fig. 1.

The invention still further relates to a synthetic peptide deduced from an intronic J sequence of a TCR.

Examples of such peptides derived from non-human animals include but are not limited to:

(a) M E N V S N P G S C I E E G E E R G R I L G S P F L

(b) M G E Y L A E P RG F V C G V E P L C

(c) M A W H

(d) M E A G W E V Q H W V SD M E C L T V

(e) M E C L T V

(f) M T V

(g) M C G S E E V F V V E S A

(h) M A C Y Q M Y F T G R K V D E P S E L G S G L
E L S Y F H T G G S S Q A V G L F I E N M I S T
S H G H F Q E M Q F S I W S F T V L Q I S A P G
S H LV P E T E R A E G P G V F V E H D I

(i) M Y F T G R K V D E P S E L G S G L E L S Y F H
T G G S S Q A V G L F I E N M I S T S H G H F Q E
M Q F S I W S F T V L Q I S A P G S H L V P E T E
R A E G P G V F V E H D I

(j) M I S T S H G H F Q E M Q F S I W S F T V L Q I S

A P G S H L V P E T E R A E G P G V F V E H D I

(k) M Q F S I W S F T V L Q I S A P G S H L V P E T E R A E G P G V F V E H D I

(l) M W W G L I L S A S V K F L Q R K E I L C

(m) M V G A D L C K G G W H C V

(n) M R E P V K N L Q G L V S

(o) M E V Y E L R V T L M E T G R E R S H F V K T S L; and

(p) M E T G R E R S H F V K T S L.

Examples of useful peptides according to the present invention derived from human sources include but are not limited to:

i) M G L S A V G R T R A E S G T A E R A A P V F V L G L Q A V

ii) M L L W D P S G F Q Q I S I K K V I S K T L P T

iii) M L P N T M G Q L V E G G H M K Q V L S K A V L T V

iv) M G Q L V E G G H M K Q V L S K A V L T V

v) M K Q V L S K A V L T V

vi) M S E C

vii) M A H F V A V Q I T V

viii) M G I C Y S

ix) M K R A G E G K S F C K G R H Y S V

x) M L T T L I Y Y Q G N S V I F V R Q H S A

xi) M Q L P H F V A R L F P H E Q F V F I Q Q L S S L G K P F C R G V C H S V

xii) M G F S K G R K C C G

xiii) M K K I W L S R K V F L Y W A E T L

xiv) M G K V H V M P L L F M E S K A A S I N G N I M L V Y Y V E T H N T V

xv) M P L L F M E S K A A S I N G N I M L V Y Y V E T H N T V

xvi) M E S K A A S I N G N I M L V Y Y V E T H N T V

xvii) M L V Y Y V E T H N T V

xviii) M E E G S F I Y T I K G P W M T H S L C D C C V I G F Q T L A L I G I I G E G T W W L L Q G V F C L G R T H C

xix) M T H S L C D C C V I G F Q T L A L I G I I G E G T W W L L Q G V F C L G R T H C

xx) M E S Q A T G F C Y E A S H S V

In still a further aspect, the invention relates to an antibody raised against a peptide as defined above. These antibodies are useful as markers of mesenchymal cells, for example for diagnostic purposes and for prognosis of cancer. According to one currently most preferred embodiment of the invention antibodies against peptide (b), are useful for diagnosis and prognosis.

Antibodies to mesenchymal TCRβ detect membrane structures, also observed following co-expression of recombinant mesenchymal TCRβ and pTα cDNAs, suggesting the existence of a unique mesenchymal TCRβ protein. Expression of mesenchymal TCRβ mRNA correlates with fast growth and tumorigenesis and is cell cycle phase dependent. Overexpression of this TCRβ along with pTα results in apoptosis, demonstrating association of these molecules with mechanisms of mesenchyme growth control.

According to the present invention mesenchymal TCR may be either directly or indirectly involved in the regulation of stem cell growth and differentiation.

Mesenchymal cells from the bone marrow are known to be obligatory for the maintenance and renewal of hemopoietic stem cells in vitro, and critical for the maintenance of hemopoiesis in vivo. In fact, the growth and differentiation of cells within different tissues, and the development of tumors, are all dependent on mesenchymal functions. It is now disclosed that the lack of TCR in mesenchymal cells causes loss of the ability of the mesenchyme to support hemopoiesis.

**BRIEF DESCRIPTION OF THE DRAWINGS**

**Fig. 1** depicts the nucleotide sequence of the $J^{int}J\text{-}C\beta_2$ mRNA transcript of the stromal/mesenchymal cell line, MBA-13, and the deduced amino acid sequence encoded thereby. The cDNA products were obtained from reverse transcription (RT)-PCR analysis using TCR primers and sequenced.

**Figs. 2A-2F** show flow cytometric analysis of $J^{int}J\text{-}C\beta_2$ expression by mesenchymal cells. Mouse embryonic fetal fibroblast (2E) and different MBA-13 cell strains (1-3; 2A-2C, respectively) were stained with preimmunized (black histogram) or immunized (green histogram) purified antibodies from rabbit serum. The rabbits were

immunized to intronic Jβ2.6 peptide (b) of the sequence M G E Y L A E P RG F V C G V E P L C. As a second antibody, we used Fab FITC conjugated donkey anti-rabbit IgG. Staining with second antibody only gave an histogram shown in blue color. Cells stained with rabbit polyclonal antibodies to irrelevant peptide 1121 of the sequence RGGGGGRGGLHD, similarly produced and purified, served as negative control (brown histogram). Competition of antibody binding was performed by pre-incubation of the purified immune serum with the specific immunizing intronic-Jβ2.6 peptide (b), for 30 min at room temperature (2D, red histogram). Competition with irrelevant peptide 1121 served as negative control (data not shown). The results of a single experiment, out of 3 performed, are shown.

**Fig. 3** shows RT-PCR analysis of the novel TCRCβ2 cDNA including an in-frame intronic J sequence designated $J^{int}J\text{-}C\beta_2$, obtained from MBA-13 mesenchymal cell line and fetal primary cell cultures. The cDNA was obtained from RNA extracted from mouse embryonic fetal fibroblast and different MBA-13 cell strains (1-3). RT-PCR was performed using the following sense pairs:

exonic Jβ2.6: 5'-CTATGAACAGTACTTCGGTC-3'; or

intronic Jβ2.6: 5'-ATGGGAGAATACCTCGCTG-3'; or

5'-CCCTAAATGGGAGAATACC; and

antisense primer Cβ3: 5'-CATCCTATCATCAGGGGGTTCTGTCTGCAA-3'.

Products of 465 bp and 524 bp were produced, respectively.

**Fig. 4** depicts sequences of all possible versions of TCRαβ containing an intronic '5 end including an in-frame Met codon as collected from available data bases: the intronic Jβ sequences Jβ2.1 and Jβ2.6, and the intronic Jα sequences JαTA31, JαTA46, JαNew05, JαS58, JαNew06, JαNew08, JαLB2A, JαDK1 and JαTA39.

**Fig. 5** shows determination of generation time of different clones of MBA-13 cell line. Eight individual clones were studied by PCR for expression of M-TCR (TCRβ $J^{int}$-$J_{2..6}C$). Out of those, four were found to be negative (M-TCR⁻ clones E4, C6, G1, B7) and four were found to be positive (M-TCR⁺ clones C4, D10, B10, B1). Cells were seeded for

different concentrations ($10^3$, $5x10^3$ and $10^4$/ml) and cell growth was determined after 44 – 46 hours. The population's generation time was calculated.

**Figs. 6A-6C** show RT-PCR analysis of TCR expression in different cell lines and primary cell cultures. cDNA was obtained from RNA extracted from different cell types, as described in the Materials and Methods section hereinafter, and RT-PCR was performed using the following primer pairs: Cβ1 and Cβ2 primers for TCRCβ2 produced a 410 bp product (Fig. 6A); Cα1 and Tm or Cα1 and Cα2 for TCRCα produced a 356 bp or 138 bp product, respectively (Figs. 6B and 6C).

**Figs. 7A-7D** show mRNA expression of TCRCβ (7A-7B) , TCRCα (7C) and CD3ε7 ) D) mRNA transcripts. Poly A+ mRNA, from mesenchymal (MBA-13, AC-6, NIH3T3, fetal cell culture), epithelial (1C8) and endothelial-adipocyte (14F1.1) cell lines, was Northern blotted as described in the Materials and Methods section hereinafter, and probed with the following probes: TCRCβ , TCRCα and CD3ε. For the TCRCβ chain, thymus RNA exhibited 1.3 kb (full-length) and 1.0 kb (truncated) transcripts, while the mesenchymal MBA-13, AC-6 and fetal cells exhibited a 1.1 kb transcript (Figs. 7A and 7B). For the TCRCα chain, thymus RNA and non-T cell lines exhibited a 1.6 kb transcript (Fig. 7C). For the CD3ε chain, thymus RNA exhibited a 1.5 kb transcript, while non-T cells showed a transcript whose size was slightly larger (Fig. 7D). Hybridization signals for TCRCβ were quantitated by densitometric scanning, and the signal value of MBA-13 was 60 fold less than thymocytes.

**Figure 8.** shows flow cytometric analysis of CD3ε, TCRαβ and TCRγδ antigens expression by MBA-13 cells. MBA-13 cells were stained with FITC-conjugated TCRαβ, CD3ε and with PE-conjugated TCRγδ (line). For intracellular staining, cells were fixed and stained with FITC-conjugated TCRαβ using the Cytoperm kit. In all experiments, cells stained with isotype-matched FITC-conjugated rat anti-mouse IgG were also prepared as negative controls (full line). The results of a single experiment are shown.

**Figure 9.** human TCR J2.3-Cβ transcript was cloned from cDNA of cord blood mononuclear cells and amniotic fluid cells:. The cloned transcripts were sequenced and

were found to be identical. The lines above the sequence indicate the boundaries of each segment. The predicted protein product is shown bellow the sequence. Bold font indicates an A to G transition that was found in both clones.

**Figure 10.** Expression of GFP-TCR J2.3-Cβ in 293T transfected cells. Western blot analysis. Each lane was loaded with lysate of $5x10^5$ cells, GFP-TCR J2.3-Cβ was detected with Anti-GFP monoclonal antibody JL-8 Clontech (Palo Alto, CA).

**Figure 11.** Mouse embryo fibroblasts from knockout (TCRβ-/-) and wild type mice, at passage 6 in vitro, were seeded at $8x10^4$ per plate into 50mm Falcon tissue culture plates and were incubated until reaching confluence. Bone marrow cells at $10x10^5$ were then seeded onto the adherent cell layers in alpha medium supplemented with 20% horse serum (selected to allow long term hemopoiesis) and $10^{-7}$M hydrocortisone. The cultures were incubated at 33°C. At the time points indicated half of the medium containing non-adherent cells was removed and new growth medium was added. The bars represent cell counts per plate at each time point. Red bars = wild type, white bars = knockout.

**Figure 12.** MEF from TCRβ-/- and wild type animals, at passage 10 were seeded as above. These cultures were then seeded with purified stem cells (1000 per plate). Other details are as in Figure 11.

**Figure 13.** μ heavy chain mRNA transcript detection by Northern blot analysis. Total RNA and poly A+ RNA samples were hybridized with μ heavy chain probe. Lane 1 mouse spleen total RNA; Lane 2 MBA 2.1 total RNA; Lane 3 MBA 2.1 poly A+ RNA.

**Figure 14.** Immunoblot analysis of m heavy chain with goat anti mouse- μ chain.

Cells lysates were loaded on 10% SDS gel and then transferred to Nitrocellulose membrane. The membrane was blotted with goat anti-mouse-μ chain. Lane 1 - 70Z cells ; Lane 2 - MBA 2.4 ; Lane 3 - MBA 2.1.

**Figure 15.** Immunostain of MBA 2.1 cells with goat anti mouse- μ chain and amplified with Biotinylated donkey anti goat and ABC kit.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to new mRNA transcripts and proteins encoded by these transcripts which are short versions of α and β TCR as detailed hereinabove in the Summary of the Invention section, and to uses of these molecules.

While studying the interactions of stromal cell lines with thymic T cells, we used reverse transcription polymerase chain reaction (RT-PCR) to amplify TCR gene fragments. Unexpectedly, the MBA-13 mesenchymal stromal cell line, derived from mouse bone marrow, was found to consistently express TCRβ constant (Cβ) region, while cDNA from a negative control tissue, i.e. liver, and from several control cell lines such as pre-B cells, plasmacytoma and mastocytoma cells, did not produce PCR products using primers from the TCR gene.

Further studies with a variety of stromal cell lines, in accordance with the present invention, showed the existence of TCR gene derived mRNAs that encode short versions of the gene consisting of the constant (C) domain, which is identical to that of T cell receptor, a joining (J) region, which may be one of several alternatives, and a 5' domain consisting of a nucleotide sequence corresponding to an intronic J sequence (again one of several alternatives) including an in-frame codon for methionine. This mRNA lacks V region sequences. One of such molecules, namely a new version of a TCRβ2.6, is shown herein to exist in mesenchymal cells and to encode a cell surface mesenchymal protein. Expression on the mRNA level has also been observed in the thymus.

According to the present invention, the uncloned stromal/mesenchymal mouse MBA-13 cell line was subdivided into substrains that either express or do not express the molecule of interest, i.e. the $J^{int}$-Jβ2.6-C protein and mRNA, on the mRNA and antigenic

protein levels. We therefore single cell cloned MBA-13 cells and obtained 8 different clonal populations by standard procedures. Out of these, 4 expressed the $J^{int}$-Jβ2.6-C protein (M-$TCR^{+}$ clones C4, D10, B10, B1) and 4 were negative (M-$TCR^{-}$ clones E4, C6, G1, B7). Fig. 5 shows that all the cells positive for $J^{int}$-Jβ2.6-C had a population generation time (doubling time) of 15 hrs or less, which is considered very fast for mesenchymal cells. On the other hand, although the negative clones showed variable results, all grew much slower and 2 clones had a very slow growth rate with doubling time between 36-38 hrs. It is therefore implied that the expression of the gene of interest correlates with fast growth rate and that lack of expression results in retarded growth. These results are supported by preliminary data indicating that antibodies to TCRβ constant region interfere with the growth of mesenchymal cells.

Thus, the expression or lack of expression of the mesenchymal TCR seems to control mesenchymal cell growth. The invention is therefore related to the use of the cDNA and antisense molecules of the invention derived from mesenchymal TCR mRNAs for expression in cells and tissues for the purpose of modulating stromal/mesenchymal cell growth and thereby their related tissue functions. For this purpose, the cDNA or antisense molecule is inserted in appropriate vectors such as, but not limited to, the retroviral vectors DCA*l* and DCM*m* that are being used in clinical trials in gene therapy (Bordignon et al., 1995). Preferably, the vector containing the cDNA or the antisense molecule, under the control of a suitable promoter such as that cDNA own promoter, will be used to infect or transfect suitable mammalian, preferably human, most preferably the patient's autologous mesenchymal cells. The thus obtained genetically-modified mesenchymal cells are then administered to a patient in need thereof by an appropriate route and are expressed in the desired site or tissue.

In one embodiment, for the treatment of wounds, local application of the cells containing the cDNA molecule can be used to induce mesenchymal cell growth thus enhancing the wound healing process. In another embodiment, mesenchymal cells of the tumor can be transfected with the antisense cDNA and then be used for treatment of localized solid tumors, to achieve regression of the tumor mesenchyme and subsequent regression of the tumor.

The proteins encoded by the mRNAs of the invention are cell surface receptors of mesenchymal cells and may probably interact with ligands presented by neighboring hemopoietic or non-hemopoietic cells. Thus, in bound or soluble form, these proteins or

the peptides derived therefrom, may have modulatory effects on cells that bear said ligands.

The proteins and peptides of the invention may be used as immunogens for production of antibodies that may be used as markers of mesenchymal cells.

Thus the invention relates also to antibodies, particularly to polyclonal antibodies to the proteins and peptides of the invention as well as proteolytic fragments thereof such as the Fab or $F(ab')_2$ fragments. These polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with said protein or peptide antigen.

The following are certain currently most preferred embodiments of human sequences according to the present invention. In these embodiments, the methionine initiating the open reading frame is shown in bold italics, the amino acids that are translated from an intronic sequence upstream to J segments are shown in *italics* and the J segments are shown in bold, three dots denote the beginning of the C1 segments.

**Homo sapiens beta gene segment**

**Jβ2.3 (bases 198551 to 198627)**
***Met*** *G L S A V G R T R A E S G T A E R A A P V F V L G L Q A V* **S T D T Q Y F G P G T R L T V L** E D L K N V F P P E V A V F E P S E A E I S H T Q K A T L V C L A T G F Y P D H V E L S W W V N G K E V H S G V S T D P Q P L K E Q P A L N D S R Y C L S S R L R V S A T F W Q N P R N H F R C Q V Q F Y G L S E N D E W T Q D R A K P V T Q I V S A E A W G R A D C G F T S E S Y Q Q G V L S A T I L Y E I L L G K A T L Y A V L V S A L V L Met A Met V K R K D S R G Stop

**Homo sapiens alpha gene segment**

**Jα2 (bases 84269 to 84334)**

L L F K Stop Stop V G P V S L C N G V T Y G ***Met*** **N T G G T I D K L T F G K G T H V F I I S...**

**Jα3 (83376. To 83437)**

L Q G I E A A Met Stop R E A H R P G E N L G S T L T G C F Q Stop S L H F L S S K Met T I T T S Stop Stop Y E I Met A R Met Stop K V I N K Stop Stop LF **Stop** N I I I I I I I E A L L I L R F T L S **Stop** R E R R I A S L G N K R C K Q Q R P K E P F R ***Met*** *L L W D P S G F Q Q I S T K K V I S K T L P T* **V G V Q Q C F Q D N L W I R D Q T Q H P A...**

**Jα6(79270 to 79331)**

Q L Q E K R H I K F P L L S V L A A L S E A P C I Stop L K S S R A R P S E C L P Q A S R V W C L Y W G A G S R H G E L L P C F S A D G K V V F S P G Y T G A K E L S S P Q P L A P A P G L Q H S G A L R T A V G D F L Q L R E Y S G G F P R *Met L P N T Met G Q L V E G G H Met K Q V L S K A V L T V* **C I R R K L H T Y I W K R N Q P Y C S S ...**

**Jα8(76346 to 76405)**

S I H G H H S C K K H V Stop L T N S Stop V W Met V K L P Stop V L S R T E T L Stop L Y Stop Stop L F Stop L E Y Stop Stop H F Y I T Q G I Q S R I F S W V L S D L L S S S N G L R K I K V K Stop Stop D Stop Met P P T T L V H A C R H R N T L S N Stop L A C D L A I L A Met A Q **Stop** Q G P I L Y R V *Met S E C* **E H R L S E T C I W N W H P T S G Q S...**

**Jα9(75756 to 75816)**

Q Y N Stop S T R A Stop L L C E L Stop R N A G Stop R H F A H R T L A L R D S L K I S S S P L F I F P I R K L R P R E V G Stop I V Stop G Q C E L G L G L E P G D P G P G A I F C D C C L V N Stop T S D R Stop E V Stop V Met L I N R K N K Stop V L Q G E Y K N V L L I T S T L V Stop A P Stop T C S P A V V **Stop** K W K E K E *Met A H F V A V Q I T V* **G N T G G F K T I F G A G T R L F V K A ...**

**B2**

**Jα11(72705 to 72765)**

VNSGYSTLTFGKGTMLLVSP

E H C Y Stop S S D V W F Stop Q K N P N I A V I P L Stop K E Q G R G F F S E S S S Stop D L S I L C Q S V L W I Q D T Y I F V S S A G P T C S A S D H L S L I C K Met R I I F K L Met A Q L K P K Stop G S G I Y A D Y **Stop** S I W L I N E G F L S F S L C R S W V E I P N T A N H F C *Met G I C Y S* **V N S G Y S T L T F G K G T M L L V S P...**

**Jα13(71282 to 71342)**

D Stop K I L E S Stop S Stop R K R Q K V W L S T S S S S D L A Stop L V N L G H S I F I Y K Met K T F N I T S D F L F Stop F C G Y I I G V Y I Y F K D K L I Y V K V F C K F L N A I H S E N I I C L Stop N K K N Y V R F R I L L T Stop E F V G S Stop Stop N S H L H V I C S P R H W **Stop** K A L S L L L K Y S G S N A T Q *Met K R A G E G K S F C K G R H Y S V* **N S G G Y Q K V T F G I G T K L Q V I P...**

**Jα14(70532 to 70583)**

S Y S Met L L K K F Stop L I E E R K I I Y K D Met S N L L N S G K Met R L C T G V D S **Stop** V K Met G V R A A I L W L V K Q D Y L V K L C K S P R K K **Stop** V S E L S R E Y H L D C S Q A F H Y I Y C T T **Met** V P **Stop** K E A F S G L I P W L S L Y

S S I K K G E S S Q S S H E G D S C ***Met*** *L T T L I Y Y Q G N S V I F V R Q H S A* **V I Y S T F I F G S G T R L S V K P...**

**Jα24(60203 to 60265)**

K T S S Y L N D R A T V V I S C H L S S A E D W V Stop P Stop V N A Stop A G G F L S L Q H L K R T P R L H Stop P Q Q S G F L P L P P G R C S S W H T P S L V S Stop K K R N **Stop** K R K G E K L I S H I ***Met*** *Q L P H F V A R L F P H E Q F V F I Q Q L S S L G K P F C R G V C H S V* **T T D S W G K L Q F G A G T Q V V V T P ...**

**Jα25(59046 to 59105)**

QKDKASPLSLGRGQGCLSSQ

A Q A G G R K L Stop G V F A E P R N T V G I T Met V R I L S L V P E P D C P C C P V S T V K W R Stop K Met S P V L D V G R S C R V L R P G V H R D L R S G D G E E G Stop K R N E K Q N H K D N T E E G F I F G K E N H K A V Stop L T L E E Met H S F G G S L L R R A L C R G K L S C **Stop** V F D A E I I T ***Met*** **Q K D K A S P L S L G R G Q G C L S S Q...**

**Jα31(51207 to 51263)**

E L G W L C S W K I S L W V Stop E C T V P S N L C V Stop G Stop A H T Y D S K S C Stop Q I R F S F G S F Met P R N A K E F Stop K L I S L A F L K E T L F A L C C R A N F S S Y H K R P E T Q R K Q K K K R K K K K T Q G E S N C P L T T V L C V W **Stop** G F T ***Met*** *G F S K G R K C C G* **N N N A R L Met F G D G T Q L V V K P ...**

**Jα36(45351 to 45411)**

K L G A V S L T C N L S I L E G Stop G R R I T Stop G Q E F K T T L G N T V R P P S L Q K I N K Stop N F F K N S Q A W Stop H A P V I L A T E E V E A G G S L V P R R S R L Q Stop A K N T P L H S S L D N K V R S C L Stop K Y I F K N I K Stop I S **Stop** R R R K E ***Met*** *K K I W L S R K V F L Y W A E T L* **C Q T G A N N L F F G T G T R L T V I P ...**

**Jα40(39930 to 39990)**

N Y K I Met S W V C L C G S Stop T G S R G E S Stop Met E Y F R G F N S H L D A Stop V L I C S L N Q T L Stop L I N Met H K D S Met R L K N F C K L G P N R S S E D F L Y E L R Y N P K **Stop** I T C R K I R G Q G L S ***Met*** *G K V H V* ***Met*** *P L L F* ***Met*** *E S K A A S I N G N I* ***Met*** *L V Y V E T H N T V* **T T S G T Y K Y I F G T G T R L K V L A ...**

**B3**

**Jα41(37899 to 37961)**

Q L L S L Stop Y L P P T F T L E P H R I V S V H A P G C S Q S R P A R R S A G H R K T P D F I T C H R A P S L R W Q I S I L I T H I T V G S G D L V S N G L ***Met*** *E E G S F I Y T I K G P W* ***Met*** *T H S L C D C C V I G F Q T L A L I G I I G E G T W W L L Q G V F C L G R T H C* **G T Q I P G M H S T S A K A P R C W S H P...**

Jα44(35064 to 35126)

L G P I T H Q V Stop Q E G F I K I K P R N R K D K E F N S Q C L Q S Stop T Stop Q L L S L N H L V S T P Stop P T E V K E G N Q Q V Met L V K Stop V S G Q S Q L P S Stop E L I L W S L G K G N A S V R A H P G C P S G R D H G E S S E Stop G S E H Q *Met E S Q A T G F C Y E A S H S V* **N T G T A S K L T F G T G T R L Q V T L ...**

Having now generally described the invention, the same will be more readily understood through reference to the following example, which is provided by way of illustration and is not intended to be limiting of the present invention.

## EXAMPLES

### MATERIALS AND METHODS

#### *Human Cell culture*

293T cell line were grown in Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal calf serum FCS) (Beth Haemek, Israel), supplemented with 20mM L-glutamine, 60μg/ml penicillin, 100μg/ml streptomycin and 50mg/L Kanamycin. Amniotic fluid cells were grown in AMF medium (Biological industries, Beit Haemek, Israel).

#### *GFP-TCRj2.3-Cβ Expression Vector*

The cDNA of human TCR J2.3-Cβ was amplified from cDNA from Amniotic fluid cells and from cord blood mononuclear cells using the sense primer 5'CCGGAATTCCATGGGGCTCTCAGCGGTGG and antisense primer 5' CGCGGA TCCCTAGCCTCTGGAATCCTTTCTC and ligated into EcoRI and BamHI digested and calf intestinal alkaline phosphatase-treated pEGFPC1 (Clontech, Palo Alto, CA). DNA sequence analysis of the GFP-TCR J2.3- Cβconfirmed the intended reading frame. Proceeding from the N to C terminus, the resulting fusion protein consists of GFP a linker sequence of 10 amino acids, and TCR J2.3-Cβ

*Transfections*

293T cells were plated at 70% confluency in 6 well plates and transfected with 1.6 μg of GFP-TCR J2.3-Cβ construct using the calcium phosphate transfection method.

*Western Blot Analysis and Fluorescence Analysis*

For immunoblot analysis, 24 hrs after transfection 5 X $10^5$ 293T cells were lyzed on ice in Tris 20mM pH 8 containing 1% Triton, 140mM NaCl, 10% glycerol, 1mM EGTA, 1.5 mM $MgCL_2$, 1mM Sodium vanadate. Cell lysates were clarified by centrifugation at 15,000 g for 10 min at 4°C, and boiled after addition of SDS-sample buffer (5% glycerol, 2% SDS, 62.5 mM Tris-HCl pH 6.8, 2% 2-mercaptoethanol, 0.01% bromophenol blue).

Extracts were subjected to 12% SDS-PAGE, blotted and probed with Anti-GFP monoclonal antibody JL-8 (Clontech, Palo Alto, CA) and secondary antibody was goat anti-mouse-HRP (Sigma). Chemiluminescent signals were generated by incubation with the ECL reagent. The gels were exposed to X-ray film.

**<u>(a) Cell lines and culture:</u>**

Several cell lines used herein in the examples originated in the inventors' laboratory or were obtained from other sources: mesenchymal MBA-13, MBA-15, 14F1.1, NIH/3T3, AC-6, AC-11 and FBMD-1 cells; control C2C12, 1C8, MPC-11 and AB-8 cells; and MC/9 mastocytoma cells.

The cell lines were cultured by standard procedures such as in DMEM containing 10% FCS or with RPMI 1640 (GIBCO) containing 7% FCS, 2 mM l-glutamine, 5 x $10^{-5}$ M 2-mercaptoethanol and 1 mM sodium pyruvate (B). Cell lines were cultured in DMEM containing 10% FCS and D-9 CM containing IL-3 and IL-4, or cultured in DMEM containing 20% FCS.

**<u>(b) Primary cell cultures:</u>**

(i) Bone marrow: Mouse bone marrow cells were obtained from femur and tibia of 1-2 week old female C57BL/6 mice. Bone marrow cells were removed aseptically by flushing culture medium through the marrow cavity using a 1ml syringe fitted with a 27-gauge needle. 1 x $10^7$ cells/ml were seeded in DMEM with 20% FCS (Bio Lab, Israel) and cultured for 4-5 days at 37°C and 5% $CO_2$ atmosphere. The plates were washed and covered with fresh culture medium. After 3 weeks, a monolayer was formed. The cells

were passaged monthly at a split ratio of 1:10 using 0.5% trypsin (Sigma, St. Louis, MO) containing 0.02% EDTA.

(ii) Fetal fibroblast: Mouse embryo were chopped in PBS solution and treated with 0.5% trypsin and 0.02% EDTA at 37°C for 15 minutes. The supernatant was collected and treated again with trypsin for 30 minutes. The cell suspension obtained was then washed a few times, resuspended in DMEM containing 10% FCS to a final concentration of $10^6$ cells/ml, and cultured for 4-5 days at 37°C and 5% $CO_2$ atmosphere. When a fibroblast monolayer was formed, it was trypsinized for 5 minutes, and the cells were washed and resuspended as indicated before. This cell suspension ($2x10^5$ cells/ml) was cultured again for 4-5 days and then collected.

(iii) Thymus and liver cells were obtained from Balb/c mice, 6-10 weeks old.

**(c) Proliferation Assay:**

Stromal cells were seeded at 1 x $10^5$ cells/ml on a 96-well round-bottom microplate (Falcon, CA) for 48 hours at 37°C in a humidified atmosphere of 10% $CO_2$ in air. The subconfluent cultures were supplemented with the relevant antibodies and incubated for an additional 48 hours. The cells were then pulsed with 1 mCi/well of [$^3$H]-thymidine (Nuclear Research Center, Negev, Israel). After 24 hours, the cells were harvested, and the incorporation of tritiated thymidine was determined. Briefly, the supernatants were aspirated, the cell monolayer was washed repeatedly with PBS to remove excess thymidine and extracted with 0.1N NaOH 0.2 ml/well. A volume of 0.1 ml of the cell extract was added to 3 ml scintillation liquid/vial (Quicksafe, A. Zinsser, Germany) and the radioactivity was counted in a liquid scintillation analyzer (1600TR, Packard, CT). [$^3$H]-thymidine incorporation reflecting the DNA synthesis was expressed as the stimulation index and was calculated as the ratio of the mean of the experimental samples to the mean cpm of the control sample. Untreated cells or cells treated with irrelevant antibody served as control.

**(d) Antibodies:**

The following monoclonal antibodies (mAbs) were used in the experiments: fluorescein isothiocyanate (FITC)-mAb anti-CD3ε (clone 145-2C11); low azide no endotoxin or FITC-conjugated hamster anti-mouse TCRβ (clone H57-597); phycoerythrin (PE)-conjugated hamster anti-mouse TCRγδ) GL-3). All antibodies were purchased

from PharMingen, San Diego, CA. Goat anti-human IgM (Kalestab, Denmark), FITC-conjugated goat anti-mouse (Sigma, Israel) and mouse anti-rat IgG (Jackson Immunoresearch Labs, West Grove, PA) served as control antibodies. Hybridoma supernatants of anti-TCRβ (clone H57-597) and anti-CD3ε (clone 145-2C11) were used for activity assays. FITC-conjugated goat anti-hamster IgG was purchased from Jackson Immunoresearch Labs. Anti-rabbit FITC Fab fragments was used as a second antibody to detect staining with rabbit polyclonal anti-peptide 1121 and anti-Jβ2.6 peptide (b) antibodies.

**(e) Flow Cytometry:**

Cells were washed with PBS without $Ca^{+2}$ and $Mg^{+2}$ containing 0.02% sodium azide and incubated for 30 minutes at 4°C with FITC-conjugated anti-mouse CD3ε (clone 145-2C11) or FITC-conjugated TCRβ (clone H57-597) or anti-Jβ2.6 peptide (b) antibodies. As second antibody for anti-Jβ2.6 peptide (b) antibody, FITC-conjugated donkey anti-rabbit IgG was used (Jackson Immunoresearch Labs). For intracellular staining, cells were fixed and stained with TCRβ using the Cytoperm kit (Serotec, UK). In all experiments, cells stained with isotype-matched control immunoglobulins were also prepared as negative controls for the surface and the intracellular staining. After washing with PBS, cells were analyzed for fluorescence with a FACScan (Becton Dickinson) with logarithmic intensity scales. In most cases, 5 x $10^3$ cells were scored using Lysis II software (Becton Dickinson).

**(f) Immunofluorescence:**

Stromal cells were seeded at $10^5$ cells/ml in chamber slides (Labtec slides: Nunc, USA) (400 ml/well) and incubated for 24 hours at 37°C in a humidified atmosphere of 10% $CO_2$ in air. The slides were washed in PBS (without $Mg^{+2}$ and $Ca^{+2}$) and were either unfixed or fixed in 3.7% paraformaldehyde in PBS for 20 minutes and permeabilized with 0.5% Triton X-100 in fixing solution for 2 minutes. The cells were washed with PBS for 5 minutes and blocked with normal sheep serum for 45 minutes and then stained with the relevant antibodies for 30 minutes. After incubation, the cells were washed with PBS, stained with the fluorescent second antibody for 30 minutes, washed, embedded in 50% glycerol in PBS and cover slips were mounted and sealed. Fluorescence was examined using a Zeiss fluorescence microscope (Zeiss, Oberkochen, Germany).

## (g) RNA Isolation and Northern Blotting:

Total RNA was extracted by Tri-Reagent (Molecular Research Center, Cincinnati, OH). For Northern blotting, poly A+ mRNA was obtained using oligo dT magnetic columns (Promega, Madison, WI). 5-30 μg mRNA were Northern blotted and probed using standard techniques with probes for the following regions: TCR Cβ, TCR Cα and CD3ε. The probes were labeled with [$^{32}$P]-dCTP by random priming (Prime-a-Gene, Promega, Madison, WI), prehybridized at 42°C in 50% deionized formamide, 2 x Denhardt's solution, 0.1% SDS, 5 x SSPE, 100 mg/ml boiled salmon sperm DNA. Hybridization was performed at the same conditions with 1 x $10^6$ cpm/ml labelled probe. Filters were washed twice with 1 x SSC, 0.1% SDS at 42°C for 30 minutes and then washed twice with 0.1 x SSC, 0.1% SDS at 55°C for 30 minutes.

## (h) PCR Analysis:

Total RNAs were reverse transcribed to cDNAs by incubating purified total RNA at 37°C for 60 minutes in the presence of MMLV reverse transcriptase. The primer pairs used for CD3ε were as follows: sense primer, 5′-TGCCCTCTAGACAGTGACG-3′ ; and antisense primer 5′-CTTCCGGTTCCGGTTCGGA-3′. The TCR derived primer pairs used were as follows:

Cβ5 : 1′-ATGTGACTCCACCCAAGGTCTCCTTGTTTG-3′;

Cβ5 : 2′-AAGGCTACCCTCGTGTGCTTGGCCAGGGGC-3′;

Cβ5 : 3′-CATCCTATCATCAGGGGGTTCTGTCTGCAA-3′ ;

Cβ5 : 5′-CATCCTATCATCAGGGGGTTCTGTCTGCAA-3′;

Cβ5 : 6′-TTCAGAGTCAAGGTGTCAACGAGGAAGG-3′;

Cα1: 5′-AAGATCCTCGGTCTCAGGACAGCACC-3′ ;

Cα2: 5′-ACTGTGCTGGACATGAAAGCTATGGATTCC-3′; or

Tm: 5′-GATTTAACCTGCTCATGACG-3′ .

For PCR, thirty cycles of amplification were carried out using the following conditions for each cycle: denaturation at 94°C for 5 minutes, annealing at 58°C for 2 minutes, and extension at 72°C for 2 minutes.

## (i) Rapid Amplification of 5′ and 3′ Ends (RACE):

5′ and 3′ RACE was performed for the cloning of the TCR Cβ chain of MBA-13 cells using the Marathon cDNA amplification kit (Clontech, Palo Alto, CA). Adaptor ligated cDNA was prepared from MBA-13 mRNA according to manufacturers' directions. Hotstart-Touchdown PCR was performed as follows: 94°C for 5 minutes (x1 cycle), 94°C for 1 minute and 74°C for 3 minutes (x5 cycles), 94°C for 1 minute and 70°C for 3 minutes (x15 cycles), 94°C for 1 minutes and 68°C for 3 minutes (x10 cycles). Specific primers were used paired to the adaptor primer of the kit. The RACE products were cloned into the pGEM-T plasmid (Promega) and transfected into *E. coli* JM109 cells (Promega). DNA was purified and sequenced using an automated DNA sequencer (Applied Biosystems 373A, New England Nuclear, Boston, MA).

**(j) Statistics:**

Data are presented as the mean ± standard error of the mean. Student's t-test was performed to determine significance.

## UNIQUE TRUNCATED TCR IN MESENCHYMAL CELLS

Figure 1 shows the nucleotide sequence of a cDNA that was cloned from the stromal/mesenchymal cell line, MBA-13, that shows a Jβ2.6 flanked by an intronic J ($J^{int}$-Jβ2.6-C).

The $J^{int}$-Jβ2.6-C mRNA encodes a putative protein that according to available literature (Irving, 1998) should be capable of being expressed on the cell surface. We therefore raised polyclonal rabbit antibodies by immunizing against a peptide sequence deduced from the Jβ2.6 intronic sequence as follows: $NH_2$-LAEPRGFVCGVE-COOH, the peptide herein designated peptide (b). For this purpose, peptide (b) conjugated to KLH was injected into 2 New Zealand rabbits using Complete Freund's Adjuvant for the first boost and Incomplete Freund's Adjuvant for additional boosts. Pre-immune serum was collected before the first boost and immune sera were collected after the additional boosts. Reactivity of the serum with the peptide (b) was tested by ELISA. The serum was purified on a peptide affinity column (eluted in 0.1M glycine pH 2.5 and dialyzed to PBS). The purified anti-peptide (b) antibody was also tested by ELISA.

The immunized rabbit serum was processed by isolating the specific antibodies using a column of the immunizing peptide (b). These antibodies were then tested for their ability to recognize various cell types: MBA-13 cell strains 1, 2 and 3, mouse embryonic

fetal fibroblasts and thymus cells as shown in Fig. 2. Whereas thymus cells were not stained (Fig. 2F), as judged by FACS analysis, two strains of the MBA-13 mesenchymal cell lines showed prominent cell surface staining by the polyclonal antibodies (Figs. 2A, 2B). On the other hand, one strain of the MBA-13 cell line was negative (Fig. 2C). A striking finding is that we found correlation between the expression of the $J^{int}$-Jβ2.6-C mRNA (Fig. 3) and the reactivity of the antiserum with the stromal cells. Thus, the two cell strains that expressed $J^{int}$-Jβ2.6-C mRNA, also reacted with the antibody whereas one of the strains that did not show any $J^{int}$-Jβ2.6-C mRNA, also did not give any signal in flow cytometric analysis using the antibodies to the intronic peptide (b) (Fig. 2C, clone 3).

The specificity of the detection of the antigen by the antiserum was further verified using competition assays with the soluble immunizing peptide (b) that reduced the ability of the antiserum to stain the cells (Fig. 2D). This strongly supports the conclusion that $J^{int}$-Jβ2.6-C protein is present on the surface of the MBA-13 cells. It is noteworthy that thymocytes do express $J^{int}$-Jβ2.6-C on the mRNA level but are not reactive with the antibody (Figs. 2F and 3). This in fact should be expected since most thymocytes express productively TCRβ and suppression of the expression of other transcripts should occur. On the other hand, in mesenchymal cells that lack recombinases, no complete TCRβ molecules are formed which allow the expression of $J^{int}$-Jβ2.6-C.

The above findings were made using a permanent cell line (MBA-13) derived in our laboratory. We further aimed to find out whether primary mesenchymal cells also express the $J^{int}$-Jβ2.6-C mRNA. As shown in Fig. 2E and Fig. 3, primary fibroblasts from mouse embryo clearly express the gene on the protein and mRNA levels.

Data base survey indicated that among the seven Jβs known, also Jβ2.1 can theoretically encode a molecule such as $J^{int}$-Jβ2.6-C. Indeed, PCR analysis using appropriate primers detected this mRNA in the MBA-13 cell line. Among the 47 possible Jαs, 9 could theoretically have a composition of intronic J with an in-frame methionine codon. These sequences are shown in Fig. 4 and include: JαTA31, JαTA46, JαNew05, JαS58, JαNew06, JαNew08, JαLB2A, JαDK1 and JαTA39. Preliminary PCR analysis indicates that at least some of these versions of the α chain also exist. In addition there are 3 possible Jα molecules initiated by a methionine from within the exonic coding region (not shown).

It is known from the T cell research field that TCRβ cannot operate as a functional receptor unless coexpressed with pTα. We therefore examined the expression of the latter in the mesenchymal cells. Indeed pTα is expressed by the MBA-13 cell line as judged by RT-PCR. Thus, these mesenchymal cells seem to express a pTα/$J^{int}$-Jβ2.6-C complex which is structurally related to a reported TCR complex containing pTα and an experimentally truncated TCR (Irving, 1998). The latter complex has been shown to be sufficient for intracellular signaling suggesting that the complex in MBA-13 is likely to be effective in signal transduction.

The study of expression of TCR was extended to a variety of stromal cell lines derived by the laboratory of the present inventors or obtained from other laboratories, as well as to primary stromal cells from the bone marrow and primary mesenchymal cells from mouse embryos. Specific stromal cell clones, but not all clones tested, expressed TCRβ. Similarly, TCRα was consistently found in particular stromal cell clones (e.g., the MBA-13 stromal cell line expressed both Cβ and Cα, whereas the MBA-15 stromal cell line did not express Cβ but was positive for Cα (Figs. 6A-6C). Similar TCR amplified PCR products were observed in cultured primary embryo fibroblasts (Figs. 6A-6C), indicating that the expression of TCR was not a bizarre characteristic of in vitro passaged stromal cell lines. Rather, TCR gene expression, as judged by PCR amplification, was common to primary mesenchymal and *in vitro* passaged cells of this origin. Indeed, bone marrow mesenchymal cells, seeded *in vitro* and selected by passaging to remove contaminating hemopoietic cells, also showed clear TCRαβ fragments of the expected sizes in PCR analysis. TCR gene expression was not found in B cells, mast cells or liver cells (Figs. 6A-6B).

As shown in Figs. 7A-7B, TCRαβ mRNA was detected in the MBA-13 stromal cell line and also in primary fetal and bone marrow fibroblast cultures. The sizes of the TCRα transcript corresponded to that found in thymic T cells, whereas the size of the mRNA detected by the TCRβ probe was about 1.1 kb as compared to 1.0 kb and 1.3 kb detected in the thymus. Of significance is that this shorter mRNA version was consistently found in different stromal cell lines, as well as in primary mesenchymal cells. A 1.0 kb mRNA species has been reported in bone marrow-derived immature precursor T cells. The relationship between the mesenchymal 1.1 kb mRNA species and that found in early bone marrow thymocytes remains to be examined.

The above data thus demonstrate that cells of mesenchymal origin do express TCR receptor complex on the mRNA level. In addition to expression of TCRαβ mRNA, expression of CD3ε, which is an essential component of the functional TCR complex, was observed (Fig. 7D). Both the size of the PCR amplified product and the mRNA detected by Northern blotting deviated slightly from the sizes detected in control T cell-derived cDNA. Flow cytometric analysis of stromal cells using an antibody to TCRαβ constant region indicated that 34% of the MBA-13 cell population was stained at low intensity fluorescence (Fig. 8). These stromal cells were negative when probed with antibodies to TCRγδ. Importantly, no TCRαβ was observed in cell lines that did not show TCRαβ mRNA. These data substantiate the above-described results using antibodies to the intronic sequence of Jβ2.6

**TRUNCATED MESENCHYMAL TCR IS ASSOCIATED WITH HEMOPOIETIC STEM CELL GROWTH CONTROL AND DIFFERENTIATION**

Since our experiments with mesenchymal cell lines indicated that these cells bear cell surface TCR molecules we reasoned that in TCR deficient (knockout) animals (TCRβ-/-) the mesenchyme should be devoid of such surface TCR structures and consequently that these cells would not be recognized by anti-TCR antibodies. We therefore obtained mouse embryo fibroblasts (MEF) from wild type and TCRβ-/- C57Bl/j6 mouse embryos at day 13.5 of gestation. We chose to study embryos rather than mature mice since the lack of TCR causes immune depression in the mice which could result in secondary effects on the mesenchyme. Immunohistochemical staining of MEF from TCRβ-/- versus wild type mice showed that indeed the former do not show surface dotted staining while MEF from wild type mice did.

Mesenchymal cells from the bone marrow are well known to be obligatory for the maintenance and renewal of hemopoietic stem cells in vitro. Moreover, these cells are critical for the maintenance of hemopoiesis in vivo. This function of the mesenchyme is not restricted to blood cells. In fact, every tissue and organ is composed of a stromal mesenchyme support that interacts with the other, tissue specific cell types. Thus, the growth and differentiation of cells within different tissues, and the development of tumors, are all dependent on mesenchymal functions. We tested whether the lack of TCR in the TCRβ-/- mouse would results in a change in mesenchymal cell function. To this end we

seeded either bone marrow cells or purified stem cells onto confluent layers of mesenchymal cells from the wild type and TCRβ-/- mice under conditions that allow long-term bone marrow cell proliferation (i.e., Dexter type long term bone marrow cultures). The proliferation of bone marrow cells in the cultures was followed during one month following initial seeding of bone marrow cells. The results presented in Figure 11 and 12 show that mesenchymal cells from TCRβ-/- passaged 6 times in culture had an inferior ability to support hemopoiesis in culture compared to wild type controls. Further passaging of the MEFs resulted in complete loss of hemopoeitic supportive ability of the TCRβ-/- MEF whereas the wild type MEF were highly active. It is concluded that the lack of TCR in mesenchymal cells causes loss of ability of the mesenchyme to support hemopoiesis. These results indicate that the mesenchymal TCR may be either directly or indirectly involved in the regulation of stem cell growth and differentiation.

## MESENCHYMAL CELLS EXPRESS OTHER MEMBERS OF THE IMMUNOGLOBULIN SUPERFAMILY

The finding that mesenchymal cells express TCR genes raised the possibility that other members of the immunoglobulin (Ig) superfamily are expressed in the mesenchyme. We screened a series of stromal mesenchymal cell lines derived in our laboratory including one subtype that shares properties with endothelial cells (MBA-2.1 cells). Using RT-PCR we discovered that the MBA-2.1 cells express an abundant amount of mouse μ chain mRNA. Northern blot analysis (Figure **11**) verifies that this is the case. More importantly, we obtained evidence that the mu chain protein is expressed by this cell line. Indeed, Western blotting detected a band that corresponds to about 50 kDa in size (Figure **12**) which is the size predicted by the mRNA sequence.

Truncated μ heavy chain (mouse) -MBA2.1

Sequence I+j2

atgggtttttgtacacccactaaaggggtctatgatagtgtgac

tactttgactactggggccaaggcaccactctcacagtctcctcaS

Protein

I+j2

MGFCTPTKGVYDSVTTLTTGAKAPLSQSPS

We deduced the latter from our experience with the TCR showing the mesenchyme expresses Ig superfamily molecules which are truncated i.e. lacking the variable part and possessing a J region preceded by an intronic sequence including a codon for methionine. PCR analysis verified that MBA-2.1 cells do express such an mRNA. In addition to Western blotting we also identified the protein using immunohistochemical staining of MBA-2.1 cells using antibodies to μ chain (Figure **13**).

We thus find that at least one type, and possibly more, of stromal cells express the Ig μ chain and may present this protein as a surface molecule. The expression of the mu chain in the mesenchyme is expected to regulate or be associated with growth/differentiation control of these cells. Thus, both TCR and μ chain, are known to be linked to the cell growth/differentiation machinery and thus can be used to control stromal functions. The TCR appears to be most abundant in mesenchymal stroma whereas the μ chain seems to be abundant in endothelial stroma.

## USE OF MESENCHYMAL TCR OR Ig IN CONTROL OF CELL GROWTH OR DIFFERENTIATION

The cDNAs encoding these molecules can be used to control cell growth by either expressing the sense or the antisense sequences, depending on the intended purpose of the treatment.

In the case of tumors the stroma enhances tumor growth. It is therefore desirable to inhibit or ultimately to block stromal growth and thus it is appropriate to apply the antisense to either of the genes in order to shut off their expression. The same thing is true for diseases such as myelofibrosis when the bone marrow becomes loaded with mesenchymal cells that block normal hemopoiesis and should be eliminated.

On the other hand it may be important to increase the expression of the TCR gene in conditions requiring more mesenchymal cells such as in improper wound healing. By the same token, the mu chain may have overlapping or complementary functions to TCR and may be used in a similar manner, for other purposes relating to endothelium, i.e. to suppress neo-vascularization in tumors on the one hand and to enhance vascularization in diseases that involve defective blood vessel formation, on the other.

Our present experiments show that the TCR affects hemopoiesis, and it is likely that the μ chain has similar or complementary functions.

Having now fully described certain preferred embodiments of this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the inventions following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

All references cited herein, including journal articles or abstracts, published or unpublished U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by reference.

Reference to known method steps, conventional method steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

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CLAIMS:

1. A cDNA molecule encoded by a T cell receptor (TCR) gene, said cDNA molecule lacking V region sequences and comprising a constant (C) domain and a joining (J) region sequences, and a 5' intronic J sequence upstream said J region sequence including an in-frame methionine codon

2. The cDNA molecule according to claim 1, encoded by a TCRβ gene.

3. The cDNA molecule according to claim 2, wherein the joining (J) gene sequence is selected from Jβ2.1 and Jβ2.6.

4. The cDNA molecule according to claim 3, wherein the joining (J) gene sequence is Jβ2.1 and said 5' intronic J sequence including an in-frame methionine codon codes for a peptide of the sequence M E N V S N P G S C I E E G E E R G R I L G S P F L as depicted in Fig. 4.

5. The cDNA molecule according to claim 3, wherein the joining (J) gene sequence is Jβ2.6 and said 5' intronic J sequence including a methionine codon codes for a peptide of the sequence M G E Y L A E P R G F V C G V E P L C as depicted in Fig. 4.

6. The cDNA molecule according to claim 5, having the nucleotide sequence depicted in Fig.1.

7. The cDNA molecule of claim 2 wherein the joining J gene sequence is the intronic Jβ2.3 gene sequence coding for the peptide:

M G L S A V G R T R A E S G T A E R A A P V F V L G L Q A V

8. A cDNA molecule according to claim 1, encoded by a TCRα gene.

9. The cDNA molecule according to claim 8, wherein the joining (J) gene sequence is selected from human or murine Jα genes.

10. The cDNA molecule according to claim 9, wherein said 5' intronic J sequence including a methionine codon is selected from the group consisting of:

(i) the intronic JαTA31 gene sequence coding for the peptide:

M A W H

(ii) the intronic JαTA46 gene sequence coding for the peptide:

M E A G W E V Q H W V S D M E C L T V

(iii) the intronic JαTA46 gene sequence coding for the peptide:

M E C L T V

(iv) the intronic JαNew05 gene sequence coding for the peptide:

M T V

(v) the intronic JαS58 gene sequence coding for the peptide:

M C G S E E V F V V E S A

(vi) the intronic JαNew06 gene sequence coding for the peptide:

M A C Y Q M Y F T G R K V D E P S E L G S G L
E L S Y F H T G G S S Q A V G L F I E N M I S T S
H G H F Q E M Q F S I W S F T V L Q I S A P G S H
L V P E T E R A E G P G V F V E H D I

(vii) the intronic JαNew06 gene sequence coding for the peptide:

M Y F T G R K V D E P S E L G S G L E L S Y F H T G G
S S Q A V G L F I E N M I S T S
H G H F Q E M Q F S I W S F T V L Q I S A P G S H
L V P E T E R A E G P G V F V E H D I

(viii) the intronic JαNew06 gene sequence coding for the peptide:

M I S T S H G H F Q E M Q F S I W S F T V L Q I S A P G S H
L V P E T E R A E G P G V F V E H D I

(ix) the intronic JαNew06 gene sequence coding for the peptide:

M Q F S I W S F T V L Q I S A P G S H
L V P E T E R A E G P G V F V E H D I

(x) the intronic JαNew08 gene sequence coding for the peptide:

M W W G L I L S A S V K F L Q R K E I L C

(xi) the intronic JαLB2A gene sequence coding for the peptide:

M V G A D L C K G G W H C V

(xii) the intronic JαDK1 gene sequence coding for the peptide:

M R E P V K N L Q G L V S

(xiii) the intronic JαTA39 gene sequence coding for the peptide:

M E V Y E L R V T L M E T G R E R S H F V K T S L; and

(xiv) the intronic JαTA39 gene sequence coding for the peptide:

M E T G R E R S H F V K T S L.

11. The cDNA molecule according to claim 8, wherein said 5' intronic J sequence including a methionine codon is selected from the group consisting of:

(i) the intronic Jα3 gene sequence coding for the peptide:

M L L W D P S G F Q Q I S I K K V I S K T L P T

(ii) the intronic Jα6 gene sequence coding for the peptide:

M L P N T M G Q L V E G G H M K Q V L S K A V L T V

(iii) the intronic Jα6 gene sequence coding for the peptide:

M G Q L V E G G H M K Q V L S K A V L T V

(iv) the intronic Jα6 gene sequence coding for the peptide:

M K Q V L S K A V L T V

(v) the intronic Jα8 gene sequence coding for the peptide:

M S E C

(vi) the intronic Jα9 gene sequence coding for the peptide:

M A H F V A V Q I T V

(vii) the intronic Jα11 gene sequence coding for the peptide:

M G I C Y S

(viii) the intronic Jα13 gene sequence coding for the peptide:

M K R A G E G K S F C K G R H Y S V

(ix) the intronic Jα14 gene sequence coding for the peptide:

M L T T L I Y Y Q G N S V I F V R Q H S A

(x) the intronic Jα24 gene sequence coding for the peptide:

M Q L P H F V A R L F P H E Q F V F I Q Q L S S L G K P F C R G V C H S V

(xi) the intronic Jα31 gene sequence coding for the peptide:

M G F S K G R K C C G

(xii) the intronic Jα36 gene sequence coding for the peptide:

M K K I W L S R K V F L Y W A E T L

(xiii) the intronic Jα40 gene sequence coding for the peptide:

M G K V H V M P L L F M E S K A A S I N G N I M L V Y V E T H N T V

(xiv) the intronic Jα40 gene sequence coding for the peptide:

M P L L F M E S K A A S I N G N I M L V Y V E T H N T V

(xv) the intronic Jα40 gene sequence coding for the peptide:

M E S K A A S I N G N I M L V Y V E T H N T V

(xvi) the intronic Jα40 gene sequence coding for the peptide:

M L V Y V E T H N T V

(xvii) the intronic Jα41 gene sequence coding for the peptide:

M E E G S F I Y T I K G P W M T H S L C D C C V I G F Q T L A L I G I I G E G T W W L L Q G V F C L G R T H C

(xviii) the intronic Jα41 gene sequence coding for the peptide:

M T H S L C D C C V I G F Q T L A L I G I I G E G T W W L L Q G V F C L G R T H C

(xix) the intronic Jα44gene sequence coding for the peptide:

M E S Q A T G F C Y E A S H S V

12. An antisense DNA molecule of any of the cDNA molecules of claims 1-11.

13. An expression vector comprising a DNA molecule according to any one of claims 1 to 11.

14. A host cell comprising a vector according to claim 13, wherein the host is a mammalian cell

15. Transfected mesenchymal human cells according to claim 14.

16. A method for inducing mesenchymal cell growth comprising the step of administering to a subject in need thereof autologous transfected mesenchymal human cells comprising a cDNA molecule according to any of claims 1 to 11, in an amount effective to induce mesenchymal cell growth.

17. A method according to claim 16 for wound healing.

18. A method for suppressing mesenchymal cell growth comprising the step of administering to a subject in need thereof autologous transfected mesenchymal human cells comprising a DNA molecule according to claim 12, in an amount effective to suppress mesenchymal cell growth.

19. A method according to claim 16 for suppression of carcinomas.

20. A polypeptide encoded by a DNA molecule according to any one of claims 1 to 11.

21. A protein capable of being expressed on the cell surface or intracellularly encoded by the nucleotide sequence depicted in Fig. 1.

22. A protein according to claim 19, having the amino acid sequence depicted in Fig. 1.

23. A synthetic peptide deduced from an intronic J sequence of a TCR.

24. The synthetic peptide according to claim 23 selected from the group consisting of:

(a) M E N V S N P G S C I E E G E E R G R I L G S P F L

(b) M G E Y L A E P RG F V C G V E P L C

(c) M A W H

(d) M E A G W E V Q H W V SD M E C L T V

(e) M E C L T V

(f) M T V

(g) M C G S E E V F V V E S A

(h) M A C Y Q M Y F T G R K V D E P S E L G S G L E L S Y F H T G G S S Q A V G L F I E N M I S T S H G H F Q E M Q F S I W S F T V L Q I S A P G S H L V P E T E R A E G P G V F V E H D I

(i) M Y F T G R K V D E P S E L G S G L E L S Y F H T G G S S Q A V G L F I E N M I S T S H G H F Q E M Q F S I W S F T V L Q I S A P G S H L V P E T E R A E G P G V F V E H D I

(j) M I S T S H G H F Q E M Q F S I W S F T V L Q I S A P G S H L V P E T E R A E G P G V F V E H D I

(k) M Q F S I W S F T V L Q I S A P G S H L V P E T E R A E G P G V F V E H D I

(l) M W W G L I L S A S V K F L Q R K E I L C

(m) M V G A D L C K G G W H C V

(n) M R E P V K N L Q G L V S

(o) M E V Y E L R V T L M E T G R E R S H F V K T S L; and

(p) M E T G R E R S H F V K T S L.

25. The synthetic peptide according to claim 23 selected from the group consisting of:

i) M G L S A V G R T R A E S G T A E R A A P V F V L G L Q A V;

ii) M L L W D P S G F Q Q I S I K K V I S K T L P T;

iii) M L P N T M G Q L V E G G H M K Q V L S K A V L T V;

iv) M G Q L V E G G H M K Q V L S K A V L T V;

v) M K Q V L S K A V L T V;

vi) M S E C;

vii) M A H F V A V Q I T V;

viii) M G I C Y S;

ix) M K R A G E G K S F C K G R H Y S V;

x) M L T T L I Y Y Q G N S V I F V R Q H S A;

xi) M Q L P H F V A R L F P H E Q F V F I Q Q L S S L G K P F C R G V C H S V;

xii) M G F S K G R K C C G;

xiii) M K K I W L S R K V F L Y W A E T L;

xiv) M G K V H V M P L L F M E S K A A S I N G N I M L V Y V E T H N T V;

xv) M P L L F M E S K A A S I N G N I M L V Y V E T H N T V;

xvi) M E S K A A S I N G N I M L V Y V E T H N T V;

xvii) M L V Y V E T H N T V;

xviii) M E E G S F I Y T I K G P W M T H S L C D C C V I G F Q T L A L I G I I G E G T W W L L Q G V F C L G R T H C;

xix) M T H S L C D C C V I G F Q T L A L I G I I G E G T W W L L Q G V F C L G R T H C; and

xx) M E S Q A T G F C Y E A S H S V.

26. An antibody raised against a peptide according to one of claims 23 -25.

27. The antibody according to claim 26 raised against a peptide in claim 24.

28. The antibody according to claim 26 raised against a peptide in claim 25.

29. Use of an antibody according to claim 26 -28 as a marker of mesenchymal cells.

30. Use of a polynucleotide or peptide sequence essentially as shown in the specification.

For the Applicants,

Cynthia Webb

Cynthia Webb

Patent Attorney

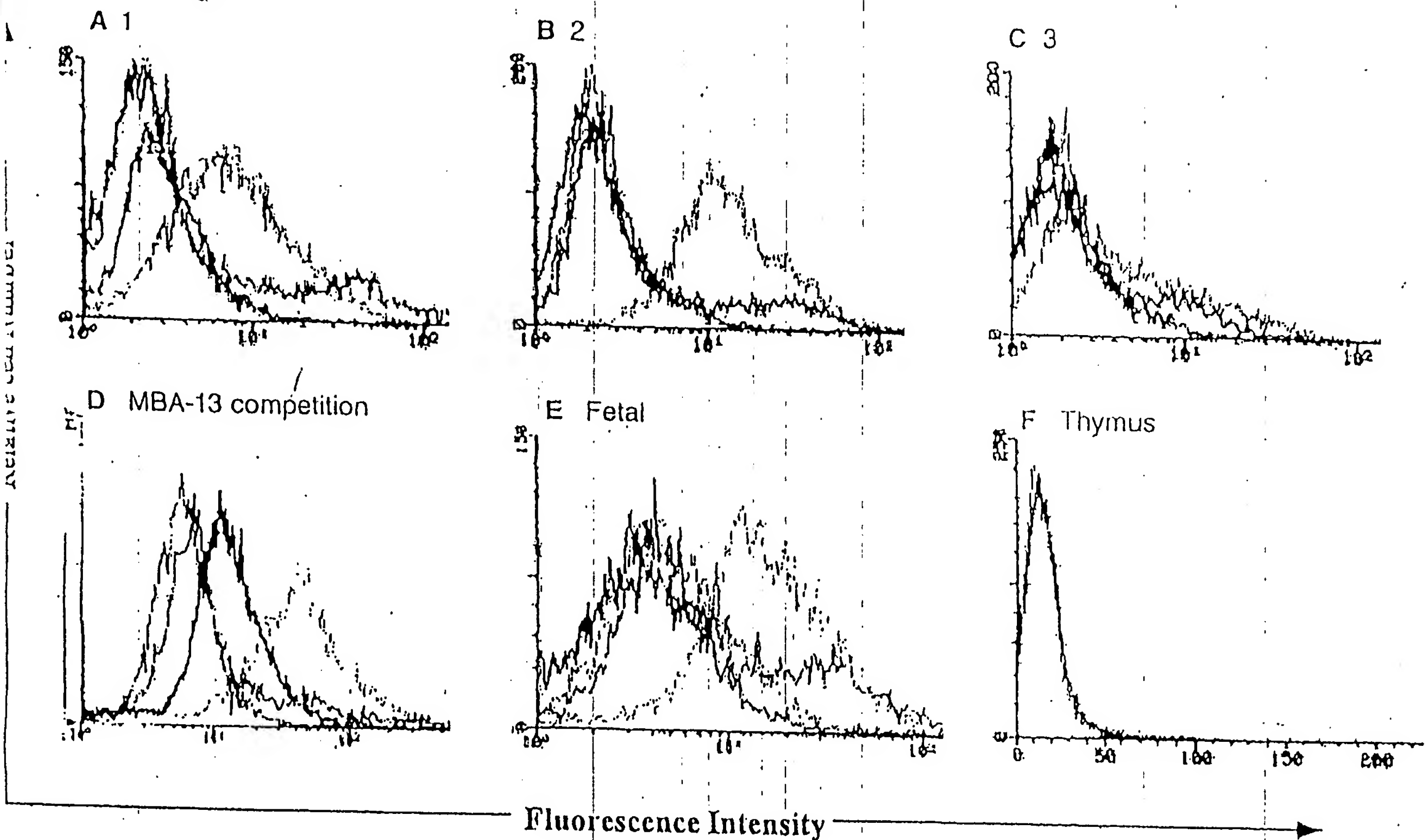
ABSTRACT

Mesenchymal cells are unexpectedly found to express specific truncated versions of T cell receptors and related proteins of the immunoglobulin superfamily, as exemplified by the mu chain. Mesenchymal T cell receptor (M-TCR) gene products either directly or indirectly controls hemopoietic stem cells. Antisense therapy can be used to prevent the proliferation of hemopoietic cells where appropriate, and antibodies could be used to reduce M-TCR function. Conversely, over-expression of the M-TCR could lead to increased hemopoiesis, for example in the processes of stem cell transplantation or following chemotherapy.

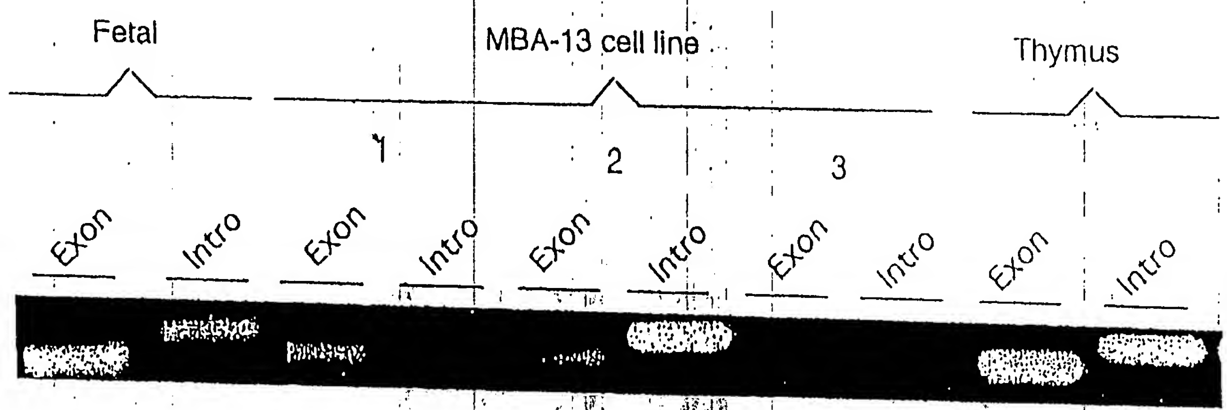
```
         |------Intron 5' to Jβ2.6 ---------------------
  1          M  G  E  Y  L  A  E  P  R  G  F  V  C  G  V  E  P  L     18
  1    TTCCCTAAATGGAGAATACCTCGCTGAACCCCGCGGGTTTGTGTGGGGTTGAGCCTC       60
       ----||-----------------Jβ2.6----------------||---Cβ2--->
 19     C  S  Y  E  Q  Y  F  G  P  G  T  R  L  T  V  L  E  D  L  R    38
 61    TGTGCTCCTATGAACAGTACTTCGGTCCCGGCACCAGGCTCACGGTTTTAGAGGATCTGA    120

 39     N  V  T  P  P  K  V  S  L  F  E  P  S  K  A  E  I  A  N  K    58
121    GAAATGTGACTCCACCCAAGGTCTCCTTGTTTGAGCCATCAAAAGCAGAGATTGCAAACA    180

 59     Q  K  A  T  L  V  C  L  A  R  G  F  F  P  D  H  V  E  L  S    78
181    AACAAAAGGCTACCCTCGTGTGCTTGGCCAGGGGCTTCTTCCCTGACCACGTGGAGCTGA    240

 79     W  W  V  N  G  K  E  V  H  S  G  V  S  T  D  P  Q  A  Y  K    98
241    GCTGGTGGGTGAATGGCAAGGAGGTCCACAGTGGGGTCAGCACGGACCCTCAGGCCTACA    300

 99     E  S  N  Y  S  Y  C  L  S  S  R  L  R  V  S  A  T  F  W  H    118
301    AGGAGAGCAATTATAGCTACTGCCTGAGCAGCCGCCTGAGGGTCTCTGCTACCTTCTGGC    360

119     N  P  R  N  H  F  R  C  Q  V  Q  F  H  G  L  S  E  E  D  K    138
361    ACAATCCTCGAAACCACTTCCGCTGCCAAGTGCAGTTCCATGGGCTTTCAGAGGAGGACA    420

139     W  P  E  G  S  P  K  P  V  T  Q  N  I  S  A  E  A  W  G  R    158
421    AGTGGCCAGAGGGCTCACCCAAACCTGTCACACAGAACATCAGTGCAGAGGCCTGGGGCC    480

159     A  D  C  G  I  T  S  A  S  Y  H  Q  G  V  L  S  A  T  I  L    178
481    GAGCAGACTGTGGAATCACTTCAGCATCCTATCATCAGGGGGTTCTGTCTGCAACCATCC    540

179     Y  E  I  L  L  G  K  A  T  L  Y  A  V  L  V  S  G  L  V  L    198
541    TCTATGAGATCCTACTGGGGAAGGCCACCCTATATGCTGTGCTGGTCAGTGGCCTGGTGC    600

199     M  A  M  V  K  K  K  N  S  *                                  208
601    TGATGGCCATGGTCAAGAAAAAAAATTCCTGAGACAAACTTTTATGCATCCTGAGCCGTT    660

661    CTTCACCCTGGCCATAGATTTTCCTGCACCTTCTCTAATTCCTGTTCCTAAGAACTTGTC    720

721    TCTTCTTCCTCCATGGATATCCATCCTTCCTCGTTGACACCTTGACTCTGAAA    773
```

FIGURE 1

GURE 2




IGURE 3



Sequences of intronic Jβ sequences containing Met:

(Green = nomenclature; M = Met; Blue = Jβ exon; ....... = continuation of Cβ2)

Jβ2.1 K G S R E V E P P F S P Y H V N H Q Q S I R T C M G N Y E L I K K H Stop V E K Stop T L C G K E V T S P F S L E A T W T P T G S L Q I S N S L C Q T L S E
Stop M D I R S Q A K S G I S S S I Stop D R P H A R S R L P Y Q F W R Stop M E N V S N P G S C I E E G E E R G R I L G S P F L L C N Y A E Q F F G P G T R L T V L ............

Jβ2.6 E L L G N C S G E F W G F W R L Y P E F P S R A L E R E A E Stop Q G D F P Stop M G E Y L A E P R G F V C G V E P L C S Y E Q Y F G P G T R L T V L......

Sequences of intronic Jα sequences containing Met:

JαTA31 V S K K K K K K K S V T I L Stop N S E P A E G A I N S S L L G S L D P G Stop N V L E H C T G L L P S P K D D P Stop C Q D R S S F L W G G G Q W I F A V I V F C L A H S P R L W Stop P E T S P Q S T T Q E Q R V K G Stop L N
Stop G E R D I G H V R T R R N F T Q K K N C H L G R C Stop S V S M A E V T P P P C P R L V S Q L R H G H Stop Q K G G F L S S L K T N L A E S H L P S S P N E P V V S V D A L G S V R R V F A V A E G S R L T R R A R W G R T Y R G W T E A S P C L H S S C A A Stop S S C G F Stop T G G R G G W G R G A I P K A V A C F G I C S G L L C L P P W E R T H L A S R R L D V A G Q E D T G V G G N S F R G E G E R G G R T V V E G V T G G S M S R M Stop S E Stop V K F K K L E I K N K K Q G R G L Q K V Y R A G T V D F V M A W H T V A N Y G N E K
I T F G A G T K L T I K P.......

JαTA46 Stop V F L P G R W E P K Stop E V D R D I S N P P C K P L V Stop L P T V D T V Stop T I Stop R T L S H I D E G S D V V H T Stop E D S R D L S L V T V S D C M P I V V H S R V Q Q T K D R D I K I R W T L S Stop P H L C N Q M I F T G S L A N G C V A Stop S L T I S P L L S P W L S F G S L S L T Stop N L K Stop
S I Y Stop I I R F L G C I T H K K M T S R H I N I N P E E R G Q R A L S Q T C S E L N L T T P C F N Q L A S A Y D Q L R Q R A T D R K W S S R H H L T R A L Stop P H Q R Stop Y F R V Q E S F P Q A G W L E R G H G S A L R Q A M E A G W E V Q H W V S D M E C L T V V T G S G G K L T L G A G T R L Q V N L.....

JαNew05 Stop V K D Stop G Y P K T K Stop V C G F A V L C S F G G C M S L P P R S L C I T L M G L C Stop L M K S G H S K D L D E E V I I I T A F F H Y Stop L R I Stop R S A Stop R Stop F I N V R L M F V L R Stop Y Stop K P N N S K I R L S
S V T Stop T H I H T H S H T H I L T H W H N H T H T H T E S Q S H T H T H S H T S T I T H T L T Q P H T H S L S L S L S L S L S L S L S L S L S L P R Q C N C I W F P S R N G C C V C L T Stop D M Q S Y Q L V S W L G F C Y C Stop F S V K T L P V K E A W C Y Q P Stop S C H Y S N H I Y T Stop P F Y Y F I S L K L A Q L I R I Q C W G N K T S G F Stop S S S E Stop L H S Q L L V L R G C S K P S Q T L G T K A A R R K A S T R G E D D V A F L G L P L G P S C L L V I V R P Q M T V N S G G S N A K L T F G K G T K L S V K S.........

JαS58 W V Stop R F H V T A V A L C S F Stop T S L L H L F Stop L E T L G F R L S F L F K K Q S L Stop S K Stop Q D L L C L L S F H I V T K A G R I C

FIGURE 4

S K L G L R L L A K V E W M Stop V Stop L V Y R K E R F V L L F F Stop P Stop
Stop Y S
K V K A T T V A S K V L Q A W S V L Q G E T W G N W L T F H G K T G M L
F V
V G L L L L L L S S L S L S L K E T Stop Y N T F Stop L S G F E Stop L G I Q
M C I T C S W Q G S R A V V L N L P N V V A P S P P K T I K L F C C Y F I A
V T L L L L Stop I G M Stop I S Y M Q L I Stop Y A T P V K G S L N P Q R R S
A L Q D E S R C C R G R W S T V S N V R G A I E L G R N T M P T F E E K K N
S S L G L E Q D Stop P L F L V S P L P L E K K P F I C N G L S R L M S F
Stop M R F H V L T Stop Stop D S L G R R S L L P L Q V Stop Stop V F Stop D
Stop V G N V N C T A K I R R A G I N S Q P L L M L S L Stop N R N Q I R M L
S S V C V H T P P R A S Stop F D Stop C Q Stop L I Q I F R H L S E Q T S L G
S L C L N Stop L S R Y L H N C Q I C F T L C C I D S A Stop Stop K Q M R L C
F P R S F S P R R S S L P P S K Stop H L F T Q R E D V Q R V T Stop L I A A
A S L H L Y D S L P W K R L K H F I R L I S Stop T D Stop Q P N Stop E E R N
R F Stop A S F L W L Q F Q A T H L E H L V R H L R N T G A R R E V V S L C G
L V F L S C T E N F T Q E E E S K Stop V E N Stop Q P G I H M Y T K Q S Stop
A S A L S G S T V W F P H S P T P A P F I S N T Y I I L F S F S F E F L S A
M P S H N P S T Y H C L S N P R M D G S G T G R V L F S G P S A E P L K K C
R L Y P S S Stop V A T R R L G R G Q D E E K P Q E S G T A S L W Stop Y I R
L N L L S G L K C F S F H L E P M C G S E E V F V V E S A T V A D R L C K C
A D I W I W H K S H S M S T......

JaNew06 K C V F S C S L G L E Q Y C S L H P Q I F S R R I Q C L A L Q T L
P V Stop P L K G S Y S F F Stop K Stop H R R I P F N V A N C G G D Stop T A Q
G P N L C S S L L Stop G Q L C L L S H R Stop T S E S G G L F P S L A F P V D
E V V L
S T N F I V K D T H D R Q L L P Y F S L N K F F L C Stop Stop L Stop Q H I S
A N E F L V I Q I N S S V T Stop T V A S Y P I I Q N S L T H H S A A A H C A
S S N P D L H A S S N K A K R M A C Y Q M Y F T G R K V D E P S E L G S G
L
E L S Y F H T G G S S Q A V G L F I E N M I S T S H G H F Q E M Q F S I W S
F T V L Q I S A P G S H L V P E T E R A E G P G V F V E H D I T V S S N T N
K V V F G T G T R L Q V L P........

JaNew08 Stop V M F H F L M F Stop N S L P L S Stop R C S E C R V G K L
H M L G H G G Q H S C T G Y S T A Q P D T T S P T T G E T A P T L P P D T K
I F L I V Y L I Stop R A K G K I K K L C P E S I L K S P R P S P P Y P H Stop S P
A D C K
F N V I F G S Y Stop K Stop Stop G F L C L M T P T V S L P S F I K G L L F C
V W P L L A S W F C P H A P L C L F Q G W A G D N S F K S H F D V T D N R
D
K V L A K C N T A H G V F S R H T T S Q L F S S V Q K H G H S Y L M S A I Y
S D T A K C S F K A G T R D F L W D L F L R L T M G W A F S G S S E M P S
W
I P A L P M E I L W S G Stop T A K P D M F L L Y R L L Q G L E I R T L R E N
K S F G Stop M G R L L D G S I R K R N D Stop Q E E R P K K N T G Q A L G
W
G G V G M S R K M V T V G I Q E A G S L S Stop E G K Q G F L Stop L K V P S
Q L S N L N Q Q G H L P F P S D F P V H V G M P L P P T M V C Stop E V G R
G
I D Q E Y V Stop H S Stop G P L F K H E T P E S V R G A K S L G P R R E M Q
Q S N S S Q Q V W R S T E Q D P V L A L C L T P L A S P D H T A H P S S F S
Stop P Q E S K V L D R E P E I P Stop P G Q V Q K G W S G A Q G W F L K T L
W I S I Stop F L I Y N K F Stop L S Stop V I R K M F L L Stop T I P V K G K
D N I Y R G P L L R C Q F P P W A S M W W G L I L S A S V K F L Q R K E I L
C L P G T G S N R L T F G K G T K F S L I P......

FIGURE 4 (CONT.)

JαLB2A Stop V I V T H P L C Stop I P P T R S I F A L S S L Stop L G S L S
N V V S V T P C P Y L L S R Y K W S K Q I L G F H Stop H S E T D N C V L D I
L Q K E G F Q S K G S H Y F Y Stop L T H K E A G D N W K V P G E Y L G F Q
K A D M A Q C M H S Stop K I P Stop L T F I E Y L L Y A C V N A P C T L S H L
R G Stop W
L W G R F Y P T F K G K V E I V T K W L R E N G G P S Stop T S S R P G C P H
C G L S Q P G S C Stop G L Y R M K Stop P V V L V T T S S V L S Q Stop P
Stop C L Stop E Q G V R Stop D S L C F L D S D T L K Q N G E C V H E Q F H S
G S M V N G Q Stop T N L K R S S L W L E S Stop P F S T P L S S L P T F L S
S W T F I S G K P L H R C L C Stop Stop R S Q I K N Stop E R L S P G H T K N
L R R Stop L F F Q Y L K N S C V D N G R G Stop H Q R Q N Q K Q Stop M K R
R
P S F S G M L L N G A V G G Q A P L Stop S L E S A L Q G L H S G S S G L R
W
R A L W K E F L W H F R L W I S C E L E V L R P H D P S I E D K R V G Y I C
F F L F L L F Stop Stop P R N R P S N C S Q A E A Y R D F F T L R R Stop R T
M I S Q C S K W G K K R R E R E R E R E R E R E R E R E R E R E R E M P Stop
R R A R G Stop T K E V G Stop L C R G Q I Stop S I E V F I S S A L E Stop N
P S I M Stop V L V T E A V F Stop T G K Q D Q G S E G L P I Stop T L S K G C
V I A F Stop Stop E R T L A V E R L L L P Q I I C L L R C S L Stop R K S D C
L P Stop L L G A W G K D L G K L R A D R R S F S A L H S Q A R E R G W G
M V
G A D L C K G G W H C V D R G S A L G R L H F G A G T Q L I V I P.......

JαDK1 Stop V C L F L W I P N L I H C Stop D K C K L F R H V S G
V S T V P I H P D I T G S K V P S H A F P V L T R K T G S S L Y C W Q A Q
Stop G S R L E D A S D A Q Q P A W D C P G R E S C S E M P S S L P L G I I L
Stop L S S P T Stop
A R P C L S V A Y S I P A S H T C G C A N I L I E A S G R S Stop G S S M L L
F Stop G K A S H Stop Stop S K A G Stop L D S P P P K S L H I P G S G L Q V
Q T T M L V F V Stop V L D M E P G C A C L Q G K H F I G Stop A I S L A H L
P V S I F F Stop E R I S W Stop Y S H L V H R Q K D D V D V P R W H T V I W
S Q A L I F P P S I F R C L S V K V I S S S M S P G G R L A C C P S S A V A
W M A S S C Y P T Stop L Stop C I P I I H L T L Y V Y L L F P Y S Stop M Y C
H A T V M L F I V S S V S S V V P I Stop T K I Q R P N C L P C L K I I V L E
K K L E F C C C L Y R H Stop E L R S L A V A R T G Y D F C S V Stop L H T P
Stop V Stop M R E P V K N L Q G L V S L C L P G R Q S S D I W N R N H G I S
Q P........

JαTA39 Stop V P D S W Stop L Stop R P P L S H S L Y H T D D H M P Y H
S S K V E L G F N E E R N Stop M L L V V A V L H P M S H S M F I I T L I T S S
D K R K F T R R T V T I C Stop T L V K M K V S T G A G A Y C N S G Y Q K D
Q A L A R K K L N K Stop Stop V D L V K L L Q I F F K N Q Y V S E L T G E Y S
A A I L S G F S Y S Y G T T V V E P C K R G F H G L N S M L S L Y S S N Q K
G G I P S R
T P K R E E S Stop M L I T S I Stop D H S R L S I F V R Q H G T T I Y N V F
I W G T R H H Stop R D A Stop Stop G C Stop D P L N L P Q Y L Stop G T V V K
E L M V H A D K H I P C M G K L S K Stop G C R T G C E Q D R S C R N P R N
N
S S R R A D P E E R A A Q L K H I Q V P Stop I C F D S C T G P A L S V K R K
C L I I L H K L I Stop G Stop V N V C K N I L Q I L K C Y P H I K Y G S I K
Q Q K I L K L G Q S Stop T L L R Stop R D G V C S C G S V A T G T G Stop K H
P L S L M E V Y E L R V T L M E T G R E R S H F V K T S L T V Q I L G L T R
G L E L G Q N S K S F Q.......

FIGURE 4 (CONT.)

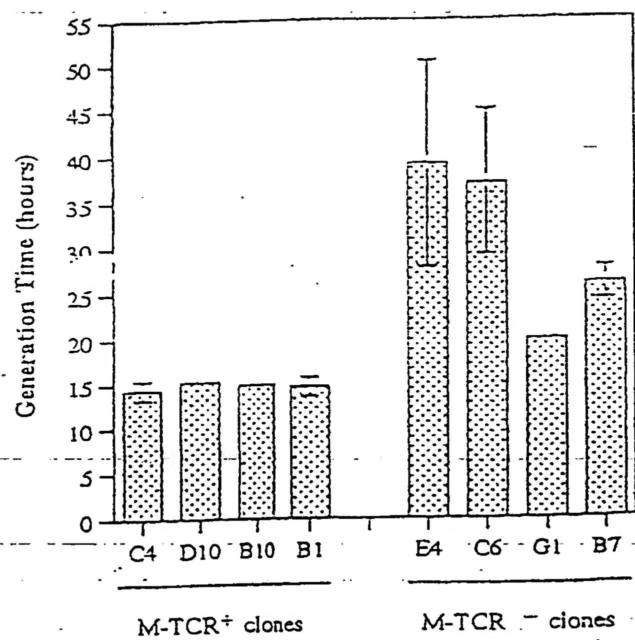

Generation Time (hours)
55
50
45
40
35
30
25
20
15
10
5
0
C4
D10
B10
B1
E4
C6
G1
B7
M-TCR+ clones
M-TCR − clones

FIGURE 5

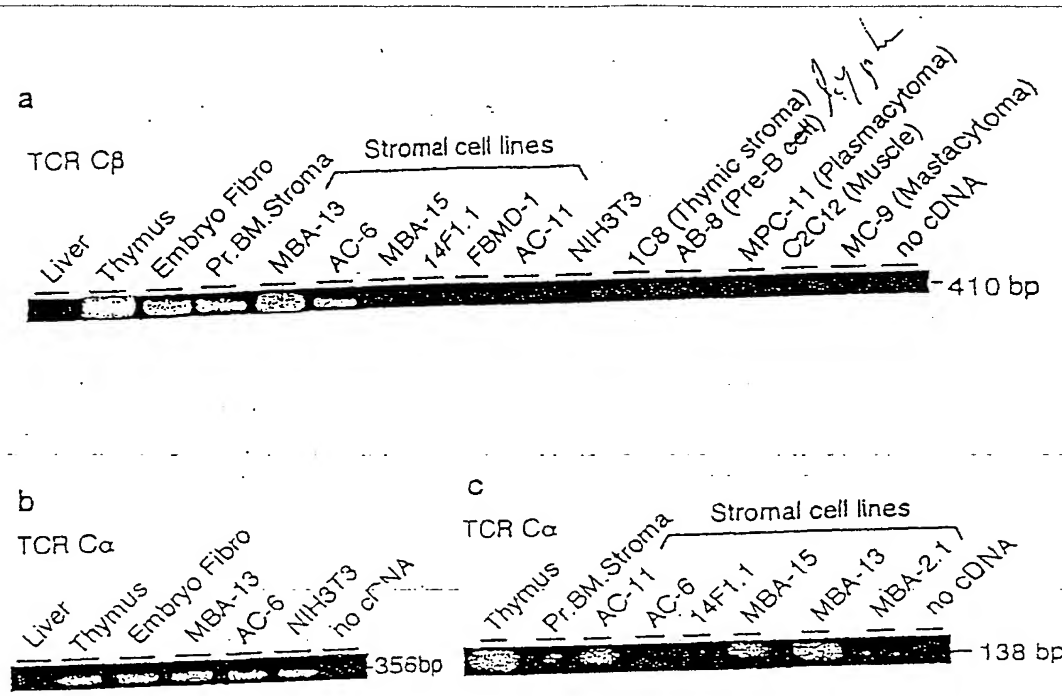

a
TCR Cβ
Stromal cell lines
Liver
Thymus
Embryo Fibro
Pr.BM.Stroma
MBA-13
AC-6
MBA-15
14F1.1
FBMD-1
AC-11
NIH3T3
1C8 (Thymic stroma)
AB-8 (Pre-B cell)
MPC-11 (Plasmacytoma)
C2C12 (Muscle)
MC-9 (Mastacytoma)
no cDNA
410 bp
b
TCR Cα
Liver
Thymus
Embryo Fibro
MBA-13
AC-6
NIH3T3
356bp
c
TCR Cα
Stromal cell lines
Thymus
Pr.BM.Stroma
AC-11
AC-6
14F1.1
MBA-15
MBA-13
MBA-2.1
no cDNA
138 bp

FIGURE 6

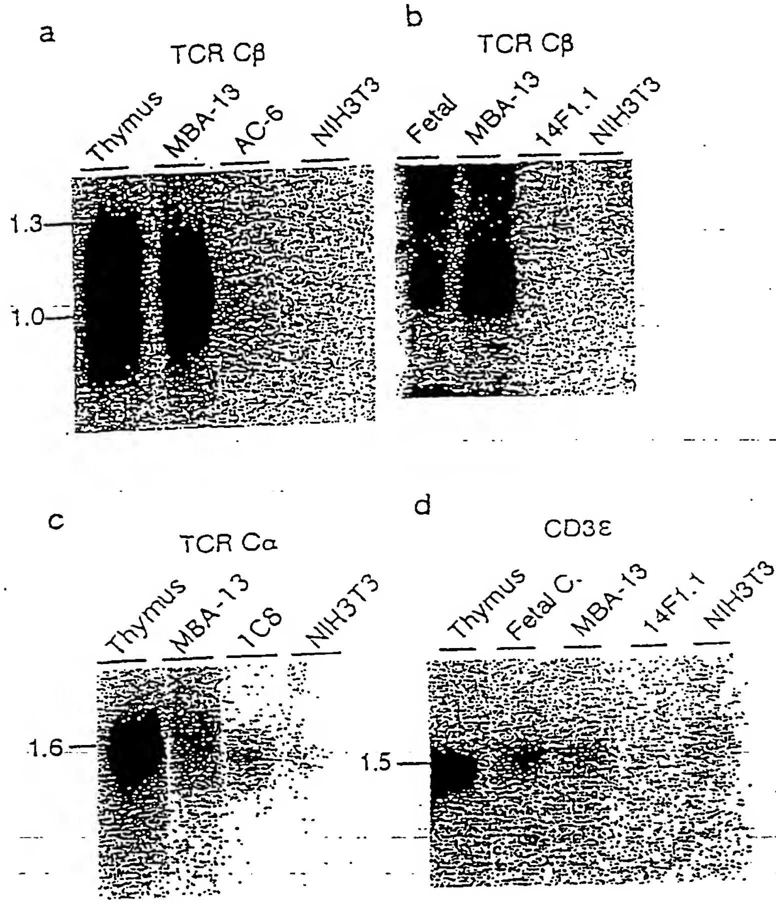

a
TCR Cβ
Thymus
MBA-13
AC-6
NIH3T3
1.3
1.0
b
TCR Cβ
Fetal
MBA-13
14F1.1
NIH3T3
c
TCR Cα
Thymus
MBA-13
1C8
NIH3T3
1.6
d
CD3ε
Thymus
Fetal C.
MBA-13
14F1.1
NIH3T3
1.5

FIGURE 7

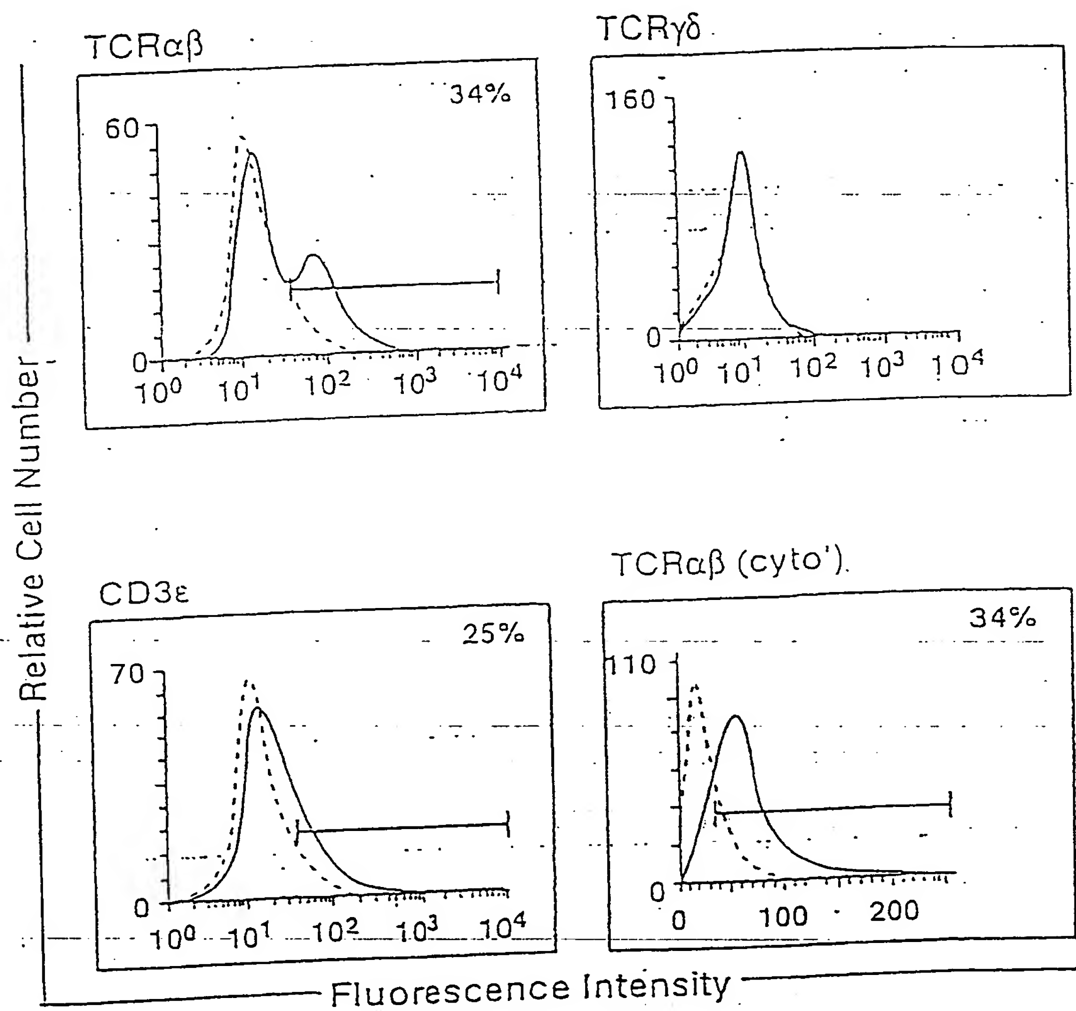

TCRαβ
34%
60
0
$10^0$ $10^1$ $10^2$ $10^3$ $10^4$
TCRγδ
160
0
$10^0$ $10^1$ $10^2$ $10^3$ $10^4$
CD3ε
25%
70
0
$10^0$ $10^1$ $10^2$ $10^3$ $10^4$
TCRαβ (cyto')
34%
110
0
0 100 200
Relative Cell Number
Fluorescence Intensity

FIGURE 8

| Intron 5' to Jβ2.3 | Jβ2.3 | Cβ2 |
|---|---|---|

```
                         Intron 5' to Jβ2.3
  1 atggggctctcagcggtgggaaggacccgagctgagtctgggacagcagagcgggcagca  60
  1  M  G  L  S  A  V  G  R  T  R  A  E  S  G  T  A  E  R  A  A   20
                                                Jβ2.3
 61 ccggttttgtccctgggcctccaggctgtgagcacagatacgcagtattttggcccaggc 120
 21  P  V  F  V  L  G  L  Q  A  V  S  T  D  T  Q  Y  F  G  P  G   40
                                           Cβ2
121 acccggctgacagtgctcgaggacctgaaaaacgtgttcccacccgaggtcgctgtgttt 180
 41  T  R  L  T  V  L  E  D  L  K  N  V  F  P  P  E  V  A  V  F   60

181 gagccatcagaagcagagatctcccacacccaaaaggccacactggtgtgcctggccaca 240
 61  E  P  S  E  A  E  I  S  H  T  Q  K  A  T  L  V  C  L  A  T   80

241 ggcttctaccccgaccacgtggagctgagctggtgggtgaatgggaaggaggtgcacagt 300
 81  G  F  Y  P  D  H  V  E  L  S  W  W  V  N  G  K  E  V  H  S  100

301 ggggtcagcacagacccgcagcccctcaaggagcagcccgccctcaatgactccagatac 360
101  G  V  S  T  D  P  Q  P  L  K  E  Q  P  A  L  N  D  S  R  Y  120

361 tgcctgagcagccgcctgagggtctcggccaccttctggcagaacccccgcaaccacttc 420
121  C  L  S  S  R  L  R  V  S  A  T  F  W  Q  N  P  R  N  H  F  140

421 cgctgtcaagtccagttctacgggctctcggagaatgacgagtggacccaggatagggcc 480
141  R  C  Q  V  Q  F  Y  G  L  S  E  N  D  E  W  T  Q  D  R  A  160

481 aaacccgtcacccagatcgtcagcgccgaggcctggggtagagcagactgtggcttcacc 540
161  K  P  V  T  Q  I  V  S  A  E  A  W  G  R  A  D  C  G  F  T  180

541 tccgagtcttaccagcaaggggtcctgtctgccaccatcctctatgagatcttgctaggg 600
181  S  E  S  Y  Q  Q  G  V  L  S  A  T  I  L  Y  E  I  L  L  G  200

601 aaggccaccttgtatgccgtgctggtcagtgccctcgtgctgatggccatggtcaagaga 660
201  K  A  T  L  Y  A  V  L  V  S  A  L  V  L  M  A  M  V  K  R  220

                                                                 678
661 aaggattccagaggctag                                           225
221  K  D  S  R  G  *
```

FIGURE 9

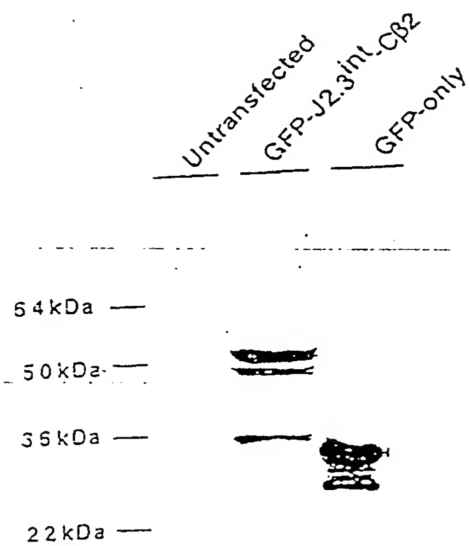

Untransfected
GFP-J2.3int_Cβ2
GFP-only
64kDa
50kDa
36kDa
22kDa

FIGURE 10

FIGURE 11

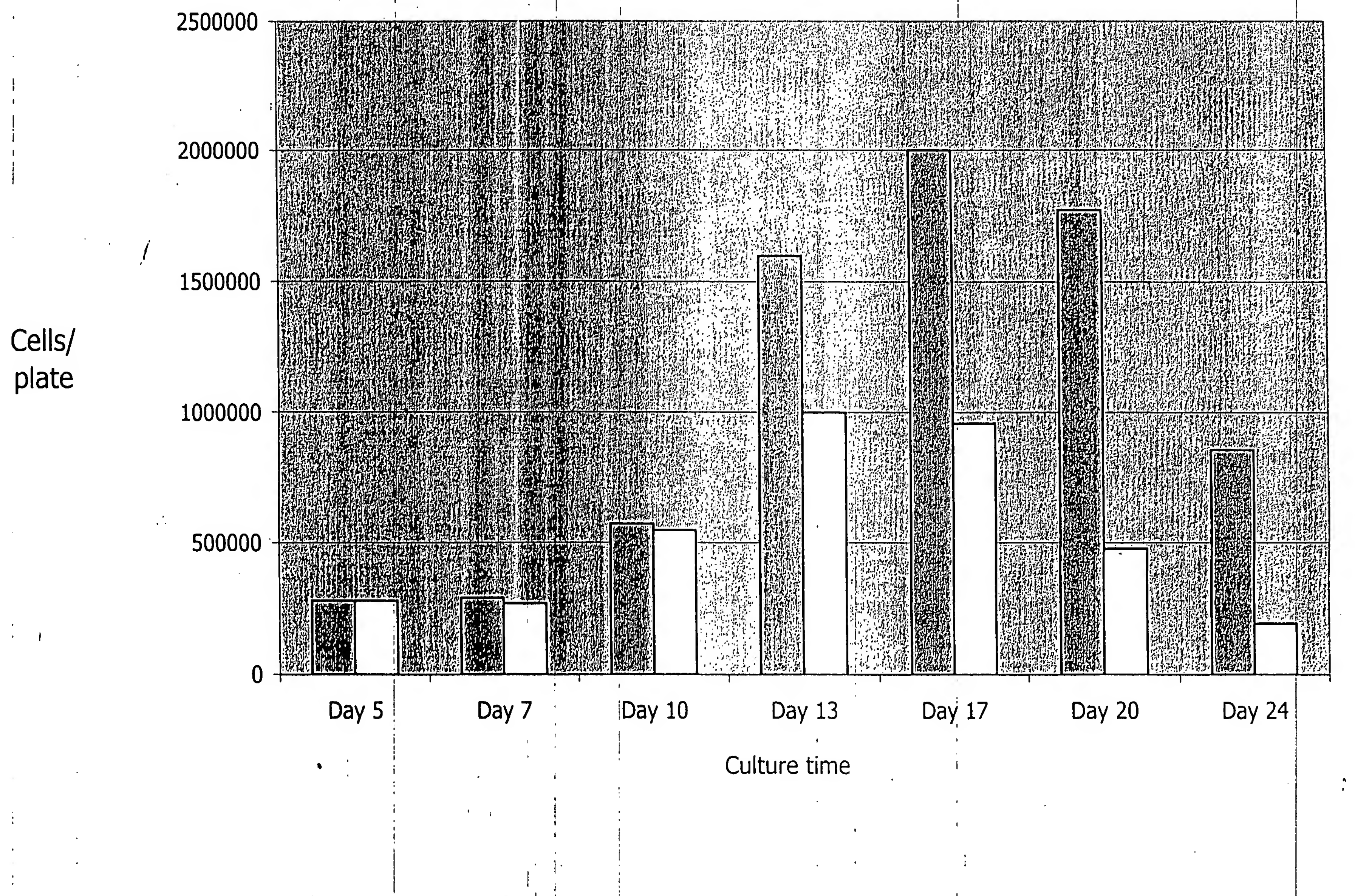

Cells/
plate
2500000
2000000
1500000
1000000
500000
0
Day 5
Day 7
Day 10
Day 13
Day 17
Day 20
Day 24
Culture time

FIGURE 12

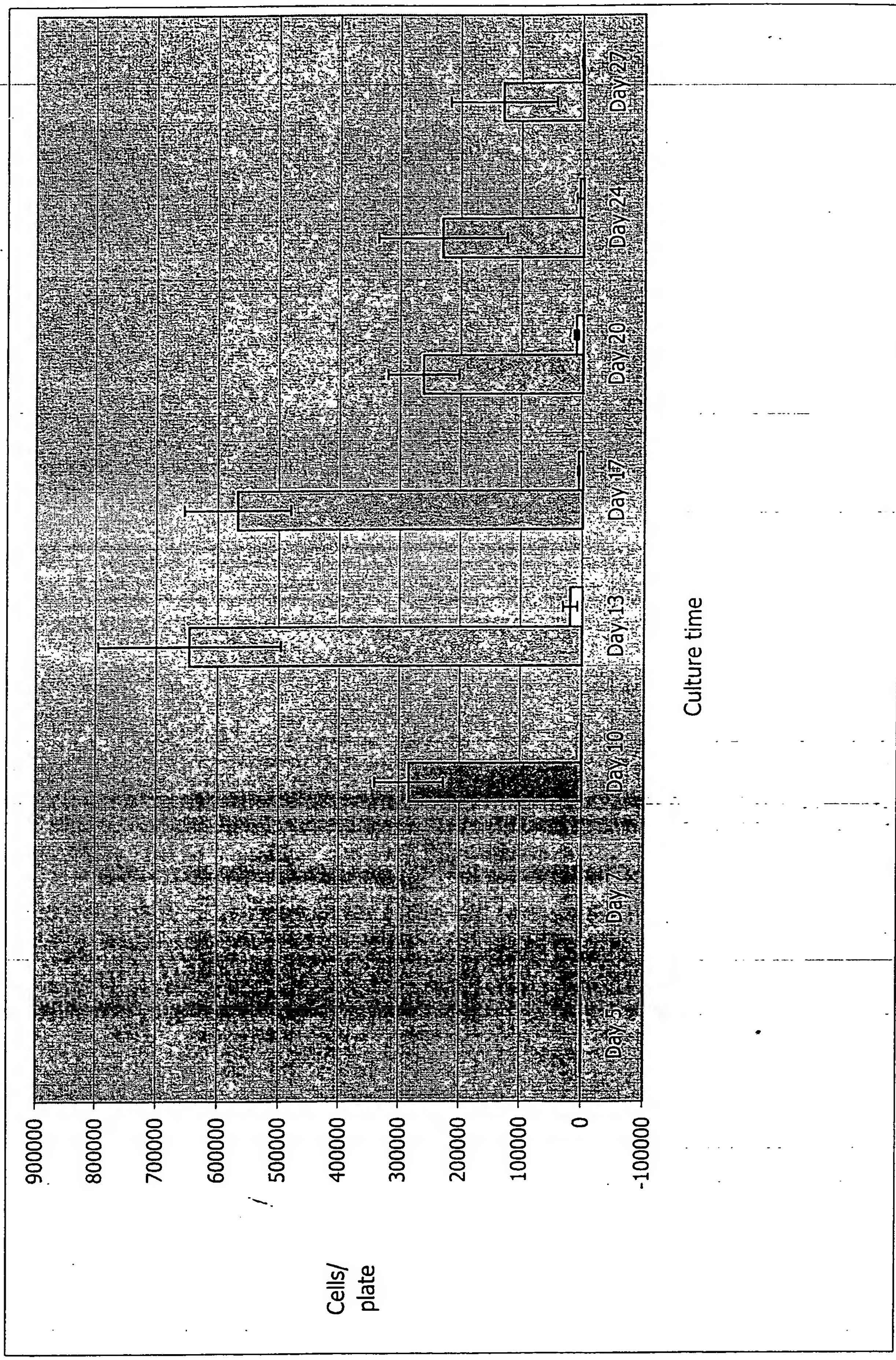
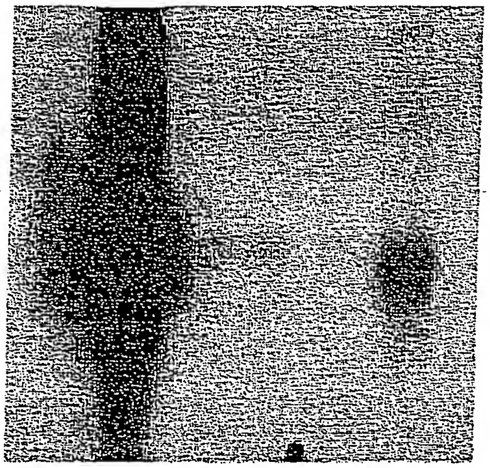

Cells/
plate
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
-100000
Day 5
Day 7
Day 10
Day 13
Day 17
Day 20
Day 24
Day 27
Culture time

FIGURE 13

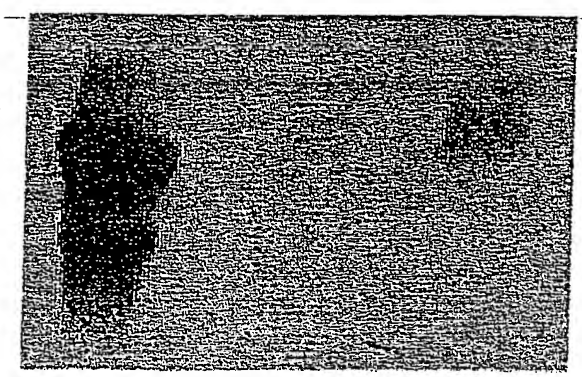
1 2 3



— 2 Kb

FIGURE 14

1 2 3



← μ

— 50 Kd

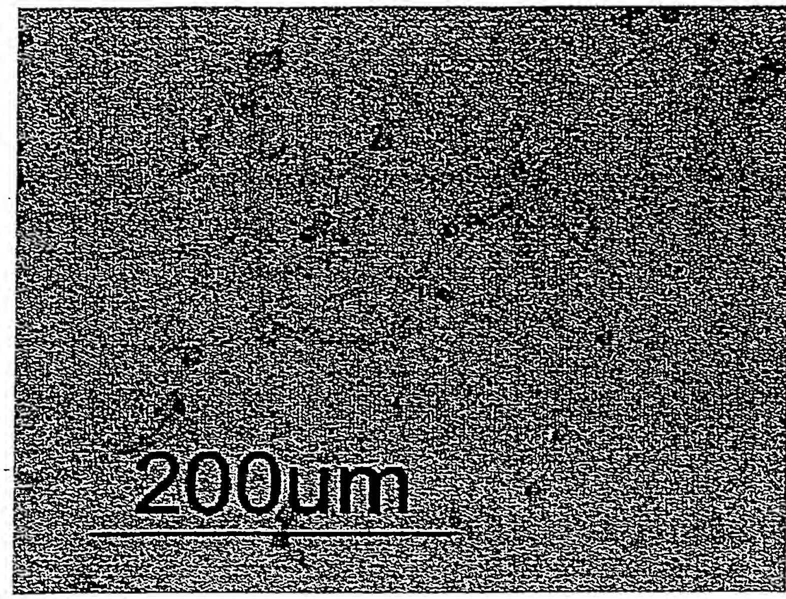



FIGURE 15